Best Available Copy

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of : Nancy Chang et al.

Serial No. : 06/659,339

Filed : October 10,1984

For : CLONING AND EXPRESSION OF HTLV-III DNA

RECEIVED
OCT 27 1997
GROUP PETITION
OFFICE OF PATENTS

Assistant Commissioner for Patents
Washington, D.C. 20231

TRANSMITTAL OF PETITION UNDER 37 C.F.R. § 1.181

Sir:

Attached is Petition to the Commissioner pursuant to 37 C.F.R. §1.181 (a) (2) and (3).

The Commissioner is hereby authorized to charge any additional fees which may be required in this application, including a petition fee, to Deposit Account No. 13-4500, Order No. 2026-4241. A DUPLICATE COPY OF THIS DOCUMENT IS ATTACHED.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

By: William S. Feiler
William S. Feiler
Reg. No. 26,728

Of Counsel:

Eugene Moroz
Mary J. Morry
MORGAN & FINNEGAN, L.L.P.
345 Park Avenue
New York, New York 10154
(212)758-4800
(212)751-6849 (FAX)

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of : Nancy Chang et al.

Serial No. : 06/659,339

Filed : October 10,1984

For : CLONING AND EXPRESSION OF HTLV-III DNA

RECEIVED
OCT 27 1997
OFFICE OF PETITIONS
A/C PATENTS

Assistant Commissioner for Patents
Washington, D.C. 20231

PETITION UNDER 37 C.F.R. §1.181

Sir:

Pursuant to 37 C.F.R. §1.181(a)(2) and (3), Petitioners request the Commissioner to personally review and reverse the denial of a petition under 37 C.F.R. § 1.182 to amend application Serial No. 06/659,339 (the "'339 Application") to add an ATCC Deposit Reference for biological material described in the '339 Application. The denial was issued on August 25, 1997.

Summary

A petition was filed to have an ATCC Deposit Reference added to the '339 Application, which is abandoned. The subject matter of the '339 Application is the subject of continuing applications still pending before the PTO. The biological material is fully described in the '339 Application and the biological

material and ATCC Deposits are fully disclosed in Petitioner's application Serial No. 06/643,306 (the "'306 Application") filed prior to the '339 Application. Petitioners respectfully submit that the addition of the ATCC Deposit Reference is a "mere technicality", whose addition is in full conformance with controlling authority. For the reasons set forth in detail below, reversal of the denial and issuance of an order granting the petition is respectfully requested.

A. The Subject of the '339 Application

1. The invention at issue is an immunoassay or diagnostic test used to detect the presence of the human immunodeficiency virus ("HIV"), the causative agent of acquired immunodeficiency syndrome ("AIDS"), in human body fluid samples, such as blood or serum.

2. HIV and the resulting AIDS conditions pose a pernicious international health threat unlike that of any other disease in modern medical history. HIV is spread by sexual contact, exposure to infected blood or blood products, and perinatal transmission from mother to child. Because many people infected with HIV remain asymptomatic for years, the development of sensitive and accurate screening methods to detect the presence of HIV in body fluids, and particularly in donated blood, has been a top priority for scientists and physicians.

3. The immunoassay of the instant invention provides a sensitive and accurate test to diagnose HIV infection. It uses

recombinantly expressed HIV proteins and body fluid samples to detect antibodies produced by the body's immune response to infection by HIV. In the assay, antibodies in the sample react with the recombinant HIV proteins and the reaction is detected. Particular types of recombinant HIV proteins are used, called envelope or "env" proteins.

B. The Human Immunodeficiency Virus

4. Like many viruses, HIV consists of a virus particle having an inner core containing genetic material and proteins, and an outer coat or envelope consisted of proteins and lipids. An organism's genetic material, often referred to as its genome, is comprised of chains of nucleic acids (either ribonucleic acid, "RNA", or deoxyribonucleic acid, "DNA"). In the case of HIV, a retrovirus, the genetic material is RNA.

5. The HIV RNA genome contains a number of genes which are the genetic "blueprint" or "code" for all of the HIV proteins and polypeptides (which are either full or partial proteins). The three main genes of the HIV genome are: 1) gag, which encodes polypeptides comprising the viral inner core; 2) pol, which encodes viral proteins called enzymes; and 3) env, which encodes polypeptides comprising the outer coat or envelope of the virus. The production of proteins based on these genetic blueprints or codes is generally called "expression".

6. To reproduce itself, HIV, like all viruses, must rely on the genetic material and machinery of a host cell which it

infects. Thus, HIV enters or infects a host cell (such as human white blood cells), releases its RNA genome, and makes a DNA copy of its genome. The DNA copy of the HIV viral genome can then become incorporated into the DNA of the host cell, which begins to replicate or make copies of the HIV genome, producing more HIV virus, thereby spreading the infection and killing the host cell.

C. The Human Immune Response to HIV

7. When HIV infects an individual, certain immune system cells called "B" cells are triggered to produce antibodies which bind to certain HIV viral proteins as part of the body's defense mechanism against infection. The antibodies formed are unique molecules that react specifically with the HIV viral proteins. Consequently, the presence of antibodies to HIV in body fluids, such as blood, indicates that the patient has been infected with HIV.

8. The viral proteins or polypeptides which elicit the production of antibodies are said to be "immunogenic". The antibodies produced in response to the HIV viral proteins bind to, or "immunoreact" with, the viral proteins. Thus, HIV viral proteins which react with antibodies are often called "immunoreactive".

D. Immunoassays

9. Immunoassays exploit the binding of antibodies to the immunoreactive viral polypeptides to detect viral infection or

the presence of virus in a sample. In general, to perform an immunoassay, an immunoreactive peptide is immobilized on a solid support, a sample of body fluid is applied and allowed to react with the polypeptide, and a detection reagent is added to indicate whether any antibodies have bound to the immunoreactive peptides, thus establishing the presence of virus or viral infection.

10. The polypeptides encoded by the env gene of a virus are very immunoreactive because they are on the envelope or outer coat of the viral particle and thus are often first exposed to the body's immune system. Consequently, env polypeptides are frequently used in immunoassays. Indeed, in 1984, those skilled in the art knew that HIV env polypeptides were immunoreactive and, thus, that HIV env polypeptides would be useful in an immunoassay.

E. Recombinant Immunoassays

11. Immunoassays for the detection of HIV antibodies can use either native viral polypeptides isolated from cells infected with live HIV virus, or recombinant viral polypeptides produced by recombinant DNA techniques. The viral polypeptides used in the immunoassay at issue are produced by recombinant DNA technology.

12. Recombinant technology enables scientists to manufacture large quantities of replicas of all or part of the virus's natural proteins through the manipulation of the virus's genetic material. Essentially, recombinant DNA technology involves the use of all or a portion of the genetic material of one organism, such as the HIV virus, to express particular polypeptides

of interest from that organism in unrelated bacterial or other host cells. For example, recombinant technology allows scientists to produce many identical copies of a particular HIV env polypeptide in bacterial cells.

13. To prepare recombinant env polypeptides for use in the immunoassay at issue, HIV genomic RNA is isolated from cell cultures infected with the virus and a DNA copy of the viral RNA is made using an enzyme called reverse transcriptase. The DNA copy of the virus is "cloned" or inserted into a DNA construct called a "cloning vector". HIV DNA fragments containing the blueprint or code for immunoreactive env polypeptides are then excised from the cloned viral genome and "subcloned" or inserted into DNA constructs called "expression vectors". Expression vectors (e.g., bacteriophage λgt11) are used to introduce the subcloned DNA fragments of interest into a host cell, which then expresses the viral polypeptides. Introduction of the expression vector into the host cell is called "transformation" of the host cell.

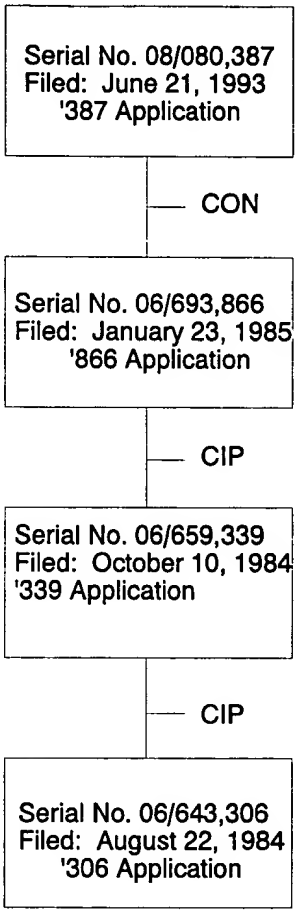
14. Once the recombinant env polypeptides have been produced by the host cell, they are isolated, purified and used in the immunoassay to detect the binding of HIV antibodies in human samples. In the immunoassay at issue, the recombinant polypeptides are immobilized on a solid support and a sample of a body fluid such as blood is applied and allowed to react. If HIV antibodies are present in the sample, they bind to the recombinant HIV polypeptides and remain attached when the support is washed. Thereafter, detection reagents, e.g. enzymes that change color, or

fluorescent or radiolabelled markers, can be used to detect the binding of the antibodies to the recombinant HIV polypeptides, indicating the presence of HIV in the sample.

15. The recombinant immunoassay has many important medical and clinical uses, including the diagnosis of patients infected with HIV and the screening of donor blood or other body fluids for the presence of HIV.

F. The Chang Patent Filings

16. The following chart illustrates the various Chang applications discussed herein:



17. Application Ser. No. 06/643,306 (the "'306 Application"), directed to Molecular Clones of the Genome of HTLV-III, was filed on August 22, 1984. [Exhibit A]. This application describes the cloning of HTLV-III from an immortalized human T-cell line and the preparation of molecular clone λBH-10. Drs. Flossie Wong-Staal, Robert C. Gallo, Beatrice Hahn and Mikulas Popovic are

the inventors. The '339 Application, Exhibit B, was co-pending with the '306 Application and shares two common inventors, namely, Drs. Gallo and Wong-Staal.[1] At the time of filing of the '306 and '339 Applications, HTLV-III was an alternative name for HIV, and is the name used in the Chang applications.

18. Prior to the filing date of all Chang applications, recombinant phage clones harboring HTLV-III DNA designated λBH-5, λBH-8 and λBH-10 were deposited by Dr. Flossie Wong-Staal, an inventor of the '339 Application. On July 30, 1984 these clones were received by the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, 20852, and accepted for deposit under ATCC accession numbers 40126, 40127 and 40125, respectively. [Exhibit C]. The deposits are in full compliance with PTO rules and are referred to in the '306 Application. The '306 Application provides a description of how to make the starting material and refers to the deposits of the starting material. [Exhibit A, page 6, lines 12-18.]

---

[1] As filed, the '339 application listed Dr. Nancy Chang as the sole inventor. On May 14, 1986, petitions to change the inventorship to add Dr. Robert Gallo and Dr. Flossie Wong-Staal were filed in the '339 application and in U.S.S.N. 06/693,866, the continuation in part application filed on January 23, 1985. Apparently, the '339 application was abandoned before the petition to change inventorship was acted upon. However, in Paper No. 13, issued November 27, 1987, the PTO examiner changed the inventorship of the '866 application to include Dr. Gallo and Wong-Staal. Pursuant to the Weil v. Fritz, 572 F.2d 856 (C.C.P.A. 1978) and In re Schmidt, 293 F.2d 274 (C.C.P.A. 1961) decisions, amendment of the '866 application was legally effective to change the inventorship of the '339 application. Thus, Drs. Chang, Gallo and Wong-Staal are the legal inventors of the '339 Application.

19. The ATCC Deposit fully complies with the PTO deposit rules. The ATCC receipt of March 6, 1987 [Exhibit C] shows that:

i) the deposit was made at the ATCC, a recognized depository;

ii) the deposit was made under the Budapest Treaty for Patent Procedures;

iii) the deposit will be released if a U.S. Patent issues citing the strain; and

iv) the deposit was made <u>before</u> the filing of the '339 Application.

20. All requirements of 37 C.F.R. § 1.808 have been fulfilled by the ATCC Deposit.

21. The '339 Application describes the cloning of HTLV-III DNA in recombinant/vector host systems capable of expressing immunoreactive HTLV-III polypeptides. [Exhibit B].

22. Application Serial No. 06/693,866 (the "'866 Application") is a continuation-in-part application of the '339 Application. The '866 Application was filed on January 23, 1985, and is pending in the Patent & Trademark Office ("PTO").

23. Application Serial No. 08/080,387 (the "'387 Application") is a continuation application of the '866 Application. The '387 Application was filed on June 21, 1993 and is pending in the PTO.

24. The '866 Application is currently involved in Interference No. 102,822 (APJ Andrew Metz). The '339 Application is currently involved in Interference No. 103,659 (APJ Michael Sofacleus).

F. The '339 Application Describes the Biological Material

25. The '339 Application discusses as an embodiment of the invention that "lambda $_{10}$ clones harboring HTLV-III DNA are cloned from the replicated form of the virus" (Exhibit B, p. 8, 33 to p. 9, 1). In the '339 Application as originally filed, the nomenclature "lambda $_{10}$ clones" refers to the recombinant phage clone BH10. The nomenclature designates the HTLV-III molecular clone BH10 inserted into bacteriophage lambda, which was used in expression of HTLV-III polypeptides and expression screening. As used in the '339 Application, "lambda $_{10}$ clones" represents an abbreviated or short-hand nomenclature for lambda BH10 or λBH10 recombinant phage clones harboring HTLV-III DNA of the molecular clone BH10 in bacteriophage lambda.

26. The '339 Application also describes the characteristics of the lambda BH10 clone, designated "λ_{10}" in Figs. 1a and 1b, at p. 9, 3-8:

> Cuts are made in the cloned HTLV-III DNA with the restriction enzyme SstI. (Figure 1a) Because there are two SstI recognition sites within the LTR of HTLV-III DNA, one LTR region is not present in the cloned DNA sequence removed from the lambda$_{10}$ vector...

27. For HTLV-III protein expression, the phage lambda gt11 is used as described and taught in the '339 specification at p. 12, 1-5 and 11-14: "The EcoRI linker ligated [HTLV-III] DNA is then treated with EcoRI... and cloned in an expression vector, [λ]gt11". (p. 12, 1-5). In addition, it is disclosed that "AIDS

patient serum was used to probe the gt11 library of HTLV-III genomic DNA..." (p. 12, 11-14).

28. The restriction maps presented in Figs. 1a, 1b, and 2 of the '339 Application show restriction enzyme sites in the genome of molecularly cloned HTLV-III which correspond to the genomic restriction enzyme map of clone BH10 depicted in Fig. 2.

29. Further, inventor Nancy Chang attested to the fact that bacteriophage lambda containing HTLV-III DNA of the genomic clone BH10 (i.e., lambda BH10 or λ_{BH10}) was used in the HTLV-III cloning and expression work described in the '339 Application (see Declaration of Nancy T. Chang ("Chang Declaration") [Exhibit D], dated February 23, 1986. In this Declaration, Chang stated that

> [t]he experimental work described in the application began at Centocor upon receipt of genomic HTLV-III DNA from the laboratories of Dr. Gallo and Dr. Wong-Staal. Dr. Gallo and Dr. Wong-Staal supplied a recombinant phage (designated λ_{BH10}) consisting of the genomic HTLV-III cDNA recombined with a phage vector. The HTLV-III cDNA insert was excised from λ_{BH10} and fragmented[,] and the subgenomic fragments were cloned and expressed in host cell systems as described in the application.

(Chang Declaration, p. 2, ¶4). This statement is supported by the '339 Application disclosure. [Exhibit B, p. 8, 32-33 to p. 9, 1-8].

G. The Patent Office Interference No. 103,659

30. On September 12, 1995, the PTO initiated an interference proceeding, No. 103,659, between Chang's '387

Application and Luciw U.S. Patent 5,156,949 (the "Luciw patent"). The Luciw patent is assigned to Chiron Corporation ("Chiron").

31. The subject matter of the interference is set forth in the Count:

> In an immunoassay to detect the presence of anti-bodies to a human immunodeficiency virus (HIV) in a human sample comprising contacting said sample with an immunogenic polypeptide comprising an amino acid sequence from the envelope (env) domain of said HIV and determining whether antibodies are bound to said immunogenic polypeptide, the improvement comprising employing as said immunogenic polypeptide a recombinant polypeptide that is the expression product of cellular hosts transformed by a heterologous expression vector comprising a DNA sequence encoding said recombinant polypeptide under the control of transcriptional and translational initiation and termination regulatory sequences functional in said cellular hosts.

32. Pursuant to 35 U.S.C. §120, Chang was given benefit of the earlier filed '866 and '339 Applications.

33. Petitioners are the senior party in the interference. Luciw (patentee) is the junior party.

H. <u>The Chiron-Abbott Litigation</u>

34. After the Luciw patent issued, Chiron brought a patent infringement action based on the Luciw patent against Abbott Laboratories, one of NIH's HIV licensees. (Chiron Corporation v. Abbott Laboratories, Civil Action No. 93 4380 MHP, U.S. District Court, N.D. Cal. (the "California Litigation")). As a defense,

Abbott asserted prior invention and relied, inter alia, on the '339 Application as evidence to establish prior invention.

35. In September 1995, the District Court for The Northern District of California (Judge Patel) granted summary judgment against Abbott on the issue of whether the '339 Application satisfied 35 U.S.C. §112 to establish prior invention. Chiron Corp. v. Abbott Laboratories, 902 F. Supp. 1103 (N.D. Cal. 1995). Judge Patel found that the '339 Application could not constitute constructive reduction to practice allegedly because it failed to meet the enablement, written description and best mode requirements of 35 U.S.C. §112. Id. at 1125-29. The basis for these holdings, as stated by the Court, were the lack of a deposit of the starting material and lack of sequence information in the '339 Application. NIH and Centocor were not parties to the California Litigation.

36. Later, the California Litigation was settled prior to trial. The August 30, 1997 dismissal order provided:

> The Settlement Agreement and this stipulation for Dismissal and Order shall have no collateral estoppel or res judicata effect as to the National Institutes of Health in Interference No. 103,659 pending in the United States Patent Office. [Exhibit E].

I. The Interference No. 103,659 Preliminary Motions

37. NIH holds the senior party position in Interference 103,659 by virtue of being accorded the October 10, 1984 filing

date of the '339 Application pursuant to 35 U.S.C. §120. Luciw's first application was filed on October 31, 1984.[2]

38. During the preliminary motion period, Chiron filed motions essentially paralleling the California court submissions.

39. Chang also filed preliminary motions in the Interference and amendments to the involved and benefit applications to "fix" the problems noted by the California Court. This entailed making amendments to the abandoned '339 Application.

J. The Chang Petition

40. Amendments of abandoned applications are processed by Petition to the PTO Commissioner under 37 C.F.R. 1.182. On February 20, 1996 as part of the preliminary motions in Interference 103,659, Chang filed a petition to amend the abandoned '339 Application to include (i) a claim under 35 U.S.C. §120 for benefit of co-pending '306 Application filed August 22, 1984, and (ii) a reference to the pre-filing date deposit of a HTLV-III recombinant phage clone lambda BH-10. An amendment to the same effect was also filed on that date. [Exhibit F].

41. The first amendment was the insertion of the following statement into the '339 Application:

---

[2] From the California litigation, it appears that Luciw will allege a reduction to practice in late September 1984. However, the Court noted that it was "deeply troubled by the absence of . . . Luciw's laboratory notebook." Chiron Corp. v. Abbott Laboratories, 902 F. Supp. 1103, 1123 (N.D. Cal. 1995). Thus, Luciw will have a proof problem in the interference.

> "This application is a continuation-in-part of U.S. application Serial No. 06/643,306, filed August 22, 1984."

42. The second amendment to the '339 Application (hereinafter the "ATCC Deposit Reference") was the insertion of the following statement:

> "A recombinant phage clone harboring HTLV-III DNA, designated λBH-10, was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, 20852 on July 30, 1984 under ATCC accession number 40125."

43. On March 29, 1996, the PTO (Abraham Hershkovitz, Director, Office of Petitioners) issued a decision granting the request to amend the '339 Application to include a claim under 35 U.S.C. §120 for benefit of the '306 Application, and dismissing the request to add the ATCC Deposit Reference. [Exhibit G].

44. On May 28, 1996, Chang filed a request for reconsideration of the March 29, 1996 decision dismissing the petition to add the ATCC Deposit Reference, with a Declaration by Flossie Wong-Staal and the amendment attached as exhibits. [Exhibit H].

45. On May 28, 1996, an interview was held with the Special Projects Examiner, Brian Hearn, to whom the PTO Commissioner had delegated authority for the Petition, to discuss the request for reconsideration. The Special Projects Examiner made requests for additional information.

46. On July 29, 1996, Chang filed a supplemental request for reconsideration of the March 29, 1996 decision dismissing the petition to add the ATCC Deposit Reference. [Exhibit I].

47. On August 25, 1997, the PTO (Charles Pearson, Patent Legal Administration) issued a decision on the request for reconsideration denying the petition to add the ATCC Deposit Reference. [Exhibit J].

REASONS FOR GRANTING THE PETITION

The petition to enter the ATCC Deposit Reference amendment in the '339 Application should be granted because the amendment is in accordance with PTO rules and practice and Federal Circuit precedent, may facilitate resolution of issues in the interferences, and is in the interest of justice.

There are at least nine errors in the decisions reached by the Commissioner's delegates which require the exercise of the Commissioner's supervisory authority by personal review and reversal of the denial of the petition:

A. The Commissioner's delegates erred in denying the Petition to amend the '339 Application to add the ATCC Deposit Reference. This amendment was proper under the authority of Sampson v. Commissioner of Patents, 195 U.S.P.Q. 136 (D.C.D.C. 1976) and In re Lundak, 773 F.2d 1216 (Fed. Cir. 1985).

B. The Commissioner's delegates erred in relying on the opinion in the California Litigation relating to the '339 Application. The plaintiffs were not a party to the California

Litigation; the opinion was not a final judgment, as the parties settled the litigation; and the dismissal order specifically exempted collateral estoppel and *res judicata* effects on the Petitioners.

C. The Commissioner's delegates erred in finding that the abandonment of the '339 Application prohibited the addition of the ATCC Deposit Reference. The Court of Customs and Patent Appeals has ruled that abandoned patent applications may be amended to include mere "technical information." Sampson v. Commissioner of Patents, 195 U.S.P.Q. 136 (D.D.C. 1996).

D. The Commissioner's delegates erred in finding that the '339 Application contained no reference to any biological material. As shown by paragraphs 25 - 29, the λBH10 biological material was described in the '339 Application.

E. The Commissioner's delegates erred in holding that the deposit had not been made in accordance with the pertinent deposit rules and patent statutes. The λBH-10 deposit was made at ATCC in full conformance with PTO requirements. [Exhibit C].

F. The Commissioner's delegates erred in finding that the ATCC Deposit Reference had to be made during pendency of the '339 Application. The controlling authority is that deposits may be made at any time prior to the issuance of a patent. See e.g., In re Lundak, 773 F.2d 1216 (Fed. Cir. 1985); Feldman v. Armstrong, 517 F.2d 1351, 1354 (C.C.P.A. 1975); In re Hawkins, 486 F.2d 569, 574 (C.C.P.A. 1973); In re Argoudelis, 434 F.2d 1390, 1394

(C.C.P.A. 1970). The '339 Application, was continued in the '866 and '387 Applications which are still pending.

G. The Commissioner's delegates erred in finding that the ATCC Deposit Reference must be examined for new matter under Section 112 of the Patent Act. Under the controlling authority a deposit reference is not new matter. In re Lundak, 773 F.2d 1216, 1223 (Fed. Cir. 1985). In Lundak, the Federal Circuit held that a post-filing date deposit of a biological material "is not new matter under 35 U.S.C. §132" and is permissible where the biological material is identified in the specification as filed. Lundak, 773 F.2d at 1223 (emphasis added). The Lundak court rejected the Patent & Trademark Office's argument "that both the deposit and its accession number are new matter," and explained:

> An accession number and deposit date add nothing to the written description of the invention. They do not enlarge or limit the disclosure. This is not the shape of new matter against which section 132 was designed to guard.

Lundak, 773 F.2d at 1223, 227 U.S.P.Q. at 95.

H. The Commissioner's delegates erred in holding the ATCC Deposit Reference to be new matter. The deposits were made in August 1984 and were fully described in Petitioners' '306 Application for which the PTO Commissioner granted the petition to add a reference under 35 U.S.C. §120. Thus, Petitioners were in full possession of the ATCC Deposit Reference.

I. The Commissioner's delegates erred in relying on laches to bar the petition. The subject matter of the '339

Application is still pending before the PTO through the '866 and '387 Applications. The amendment is a mere technicality and is timely under Sampson v. Commissioner of Patents, 195 U.S.P.Q. 136 (D.C.D.C., 1976). Moreover, the petition was filed in a timely manner in accordance with the motion period set by the Patent and Trademark Board of Appeals and Interferences and within a reasonable time from the announcement of the California Litigation opinion. Since the amendment to add the ATCC Deposit Reference is a technical addition which became necessary as a result of the interference, and does not involve examination, the petition was timely filed.

ANALYSIS

Under the authority of Sampson v. Commissioner of Patents, 195 U.S.P.Q. 136 (D.C.D.C., 1976), entry of the amendment to the '339 Application is appropriate. The amendment to the application adding the reference to the deposit of the HTLV-III clone at the ATCC is also proper under In Re Lundak, 773 F.2d 1216 (Fed. Cir. 1985). As the Court noted:

> Constructive reduction to practice does not turn on the question of who has possession of a sample, and thus it does not turn on the inclusion or absence, in the specification as filed of the name and address of who will have possession of the sample on grant of the patent.
>
> * * *
>
> We conclude that .... the insertion of depository data after filing is not new matter under 35 U.S.C. § 132.

773 F.2d at 1223. The Court of Appeals further noted:

> [T]he function of section 112 in ensuring *complete public disclosure* is only violated if the *disclosure* is not complete *at the time it is made public* i.e. at the issue date.

773 F.2d at 1223 (citations omitted).

The entry of the ATCC Deposit Reference is warranted in equity to address the starting material finding, the written description issue and the best mode finding in the California Decision, which Chiron has raised in the interference. The California findings are erroneous, particularly in light of the following facts:

(1) the ATCC Deposit had been made;

(2) the ATCC Deposit could be referenced up until the issuance of a patent;

(3) the detailed description of the starting material in the '306 Application (and '339 Application);

(4) the ATCC Deposit Reference in the '306 Application; and

(5) the ability of Chang to add to the '339 Application a Section 120 reference to the '306 Application (which has been granted by the PTO).

The entry of the amendment is fully warranted under controlling law. Accordingly, entry of the proposed amendment is fully justified.

Addition Of A Reference To A Deposit Is Proper Where The Biological Material Is Specifically Identified In The Specification As Filed

First, Petitioners respectfully submit that, contrary to the position taken by the PTO, there is no requirement in either the PTO rules, the manual of patent examining procedure ("MPEP") or In re Lundak, 773 F.2d 1216 (Fed. Cir. 1985) that a specification as filed must refer to a deposit of a biological material in order to add a reference to the date, depository name and accession number of the deposited material. Indeed, the deposit rules, 37 CFR §§1.801-1.809, and the MPEP make clear that a post-filing date deposit may be made and/or a reference to deposit data added as long as the biological material was specifically identified in the Application as filed. The PTO rules state:

> Whenever a biological material is specifically identified in an application for patent as filed, an original deposit thereof may be made at any time before filing the application for patent or, subject to §1.809, during pendency of the application for patent.

37 CFR §1.804(a) (emphasis added). See also 37 CFR 1.809(d) There is no requirement that the deposit be referenced in the specification. As the MPEP explains:

> 37 CFR 1.804(a) specifies not only a permissible time frame for making an original deposit, but also specifies that the biological material deposited must be specifically identified in the application as filed. The requirement for a specific identification is consistent with the description requirement of the first paragraph of 35 USC 112 and provides an antecedent basis

> for the biological material which either has been or will be deposited before the patent is granted.

MPEP §2406.01 (emphasis added). Thus, while the biological material must be identified and referenced in the specification, the existence of a deposit need not be mentioned.

Indeed, the MPEP specifically distinguishes between the permissible addition of a reference to a deposit of an identified biological material and the impermissible addition of a reference to the biological material itself, which is prohibited as new matter under 35 USC §132:

> It should be noted, however, that reference to a biological material present in an application upon filing, may form the basis for making a deposit, where required, after the filing date of a given application, but that the reference to the biological material, itself, cannot be added after filing without risking the prohibited introduction of new matter.

MPEP §2404.03 (emphasis added).

In the instant situation, Petitioners seek only to add a reference to the pre-filing date deposit of a biological material, clone λBH-10, which was specifically identified in the specification as filed. Indeed, as discussed above and in the Declaration of Dr. Flossie Wong-Staal ("Wong-Staal Declaration", attached as Exhibit K), λBH-10 clones, which were referred to in the '339 specification as "lambda 10 clones" were specifically identified and described in the '339 specification on page 3, lines 28-30, page 8, lines 33 to page 9, line 1, and page 9, lines 3-8 of

the '339 Application as filed. Additionally, restriction maps of clone λBH-10, showing restriction enzyme sites present in this clone are found at Figs. 1a, 1b and 2 of the '339 Application as filed. See Declaration of Dr. Wong-Staal, ¶10.

Moreover, Dr. Wong-Staal's declaration makes clear that the λBH-10 clones deposited more than two months before the filing date of the '339 Application are the same as those identified and described in the '339 specification. Finally, the Wong-Staal declaration and Amendment establish that the deposit of clone λBH-10 was made in full compliance with the deposit rules of the PTO, 37 CFR §§1.801-1.809. See Exhibit K, Wong-Staal Decl., ¶¶5-8. The ATCC deposit receipt also supports this. Thus, pursuant to 37 CFR §1.802, 1.804 and 1.809(d) and MPEP §§2406.01 and 2404.03, amendment of the '339 specification to include a reference to the deposit of clone λBH-10 is proper.

Petitioners note that the August 25, 1997 decision ("the Decision") appears to interpret the Lundak case to authorize addition of deposit data to a specification only where the deposit itself was referenced in the specification. Petitioners' respectfully submit that this is an improperly narrow interpretation of Lundak. As discussed above, such an interpretation would be contrary to the deposit rules and the MPEP. Additionally, the issue before the Federal Circuit in Lundak was not whether Lundak could "update" or "clarify" his deposit data. Rather, as the Federal Circuit noted, the PTO had argued "that a post-filing deposit is barred as new matter, as is the insertion

into the specification of reference to such deposit." 773 F.2d at 1222. The Federal Circuit rejected this argument, holding that "the insertion of depository data after filing is not new matter under 35 USC §132." Thus, Petitioners respectfully submit that it is improper to interpret Lundak to authorize only the clarification or updating of deposit data.

Addition Of The Reference To The Deposit Is Not New Matter Under 35 USC §132

Second, Petitioners respectfully submit that the position taken in the Decision (p. 9-11), that amendment of the '339 specification is improper because it may constitute new matter and thus be precluded by 35 USC §132, is contrary to the controlling law.

In In re Lundak, 773 F.2d 1216 (Fed. Cir. 1985), the Federal Circuit was faced with the issue of whether a post-filing date deposit of a biological material and amendment of the specification to reference such deposit was new matter under 35 USC §132. The court concluded that the post-filing date deposit was proper and that "the insertion of deposit or data after filing is not new matter under 35 USC §132." Lundak, 773 F.2d at 1223 (emphasis added). The court explained:

> An accession number and deposit date add nothing to the written description of the invention. They do not enlarge or limit the disclosure. This is not the shape of new matter against which section 132 was designed to guard.

Lundak, 773 F.2d at 1223 (emphasis added). See also MPEP §2406.01. ("The [Lundak] court further held that the addition of information designating the depository, accession number, and deposit date of the deposited cell line in ATCC after filling date did not violate the prohibition against new matter in 35 USC 132.")

In light of the Federal Circuit's explicit holding that addition of a reference to a post-filing date deposit does not constitute new matter, Petitioners respectfully submit that addition of a reference to the deposit of clone λBH-10, made two months before the filing date, cannot be considered new matter.

Petitioners note that, based on the California Litigation opinion, the PTO questioned whether the '339 specification satisfied the enablement and best mode requirements of 35 USC §112. Petitioners respectfully submit that reliance on the California Litigation opinion is improper for several reasons. First, neither Petitioners nor their assignees were parties to the California Litigation. Consequently, Petitioners had no opportunity to be heard or to defend against Chiron's allegations regarding their specification. See, e.g., 37 C.F.R. §1.683(a). Thus, any decision in the California Litigation cannot be *res judicata* or collateral estoppel as to Petitioners, nor may it preclude or estop Petitioners from defending their applications. In dismissing the California Litigation the Court specifically provided that there would not be a *res judicata* or collateral estoppel effect on the Petitioners. [Exhibit E]. Additionally, as stated in the opinion of the Northern District of California Court, the Judge considered

the record before her "quite weak" and made her decision without benefit of expert testimony on these issues. 902 F.2d at 1126.

Second, the issue of whether the '339 Application is enabling without reference to the deposit of clone λBH-10 is irrelevant to the instant petition. 37 CFR §1.802(b) states that "[i]f a deposit is necessary, it shall be acceptable if made in accordance with these regulations." As discussed, the deposit of clone λBH-10 was made in full compliance with the deposit rules, which do not require proof that a specification is enabling without the deposit as a prerequisite for allowing the post-filing date addition of a reference to a deposit.[3] Moreover, the discussion in Lundak about whether the specification as filed was enabling was necessitated by Lundak's appeal of the rejection of his claims under 35 USC §112, not because it was a requirement to allow the addition of a post-filing date reference to his specification. See e.g., 773 F.2d at 1220.

Because the deposit of λBH-10 is in full compliance with the governing law and the addition of a reference to the pre-filing date deposit of λBH-10 "is not the shape of new matter against which section 132 was designed to guard," Lundak, 772 F.2d at 1223, Petitioners respectfully submit there is no need to examine the

---

[3] Indeed, 37 CFR §1.802(c) states:

> The reference to a biological material in a specification disclosure or the actual deposit of such material by an applicant or patent owner does not create any presumption that such material is necessary to satisfy 35 USC 112 or that deposit in accordance with these regulations is or was required.

'339 Application for new matter, and thus, 35 USC 132 does not bar entry of this amendment. The Amendment Is Permitted. The proposed ATCC Deposit Reference amendment is akin to the request to add a section 120 reference previously allowed by the Office of Petitions and is fully within the scope of amendments authorized by Sampson v. Commissioner of Patents, 195 U.S.P.Q. 136 (D.D.C. 1976).[4]

Petitioners are not seeking "to change the invention disclosed or to introduce a concept not previously present in the specification of an abandoned application or to continue prosecution of an abandoned application". Rather, Petitioners are seeking to add the ATCC Deposit Reference for an HIV clone which was specifically identified in the specification as filed and was deposited two months prior to the filing date. See Wong-Staal Decl. ¶¶ 4, 10. [Exhibit K]. As such, Petitioners respectfully request that the amendment be entered.

CONCLUSION

Petitioners respectfully request that the March 29, 1996 Decision be reconsidered, that the petition be granted and the amendment to the '339 Application be entered to protect Petitioners' rights in this important invention.

---

[4] Applicants respectfully submit that Dart Industries, Inc v. Banner, 636 F.2d 684, 687-688 (D.C. Cir. 1980), cited in the Decision at p. 10, is inapposite because reference to a biological material specifically identified in the specification is not new matter under Lundak and the PTO Deposit rules.

AUTHORIZATION

The Commissioner is hereby authorized to charge any additional fees which may be required in this application, including a petition fee, to Deposit Account No. 13-4500, Order No. 2026-4241.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

By: William S. Feiler
William S. Feiler
Reg. No. 26,728

Of Counsel:

Eugene Moroz
Mary J. Morry
MORGAN & FINNEGAN, L.L.P.
345 Park Avenue
New York, New York 10154
(212)758-4800
(212)751-6849 (FAX)

James C. Haight
NATIONAL INSTITUTES OF HEALTH
Office of Technology Transfer
Suite 325
6011 Executive Blvd.
Rockville, MD 20852
(301) 496-7056

SN 643,308
filed 8/22/84

APPLICATION FOR UNITED STATES PATENT

Inventors: FLOSSIE WONG-STAAL
ROBERT C. GALLO
BEATRICE H. HAHN
Mikulas Popovic

Title: MOLECULAR CLONES OF THE GENOME OF HTLV-III

Abstract of the Disclosure

Disclosed is the molecular cloning of HTLV-III, the adult leukemia and acquired immune deficiency syndrome (AIDS) virus. Clone BH10 contains a 9.0 Kb viral insert constituting the entire HTLV-III genome. Clones BH8 and BH5 contain viral inserts of 5.5 Kb and 3.5 Kb, respectively. These clones are suitable for the development of diagnostic and therapeutic measures for AIDS, as well as use as probes for the detection of AIDS.

In related inventions, HTLV-III was detected, isolated, and immortalized in an HT cell line. Since evidence now strongly indicates that HTLV-III is related to acquired immune deficiency syndrome (AIDS), the ability to enhance production of the virus and determine the DNA sequences of the virus is critically important to developing a cure or reagent active against AIDS. The present invention takes one such significant step by disclosing the process for molecularly cloning the complete genome of the HTLV-III virus. In short, The molecular cloning of the complete genome of the HTLV-III virus produced by one of these lines designated H9/HTLV-III is disclosed. Two forms of this virus are identified which are highly related but differ in several restriction enzyme cleavage sites. Both variants exist as integrated and unintegrated forms in the infected cell line. The complete genomes of two forms of HTLV-III are molecularly cloned and shown to exist in the long-term infected cell line both as polyclonally integrated provirus and as unintegrated viral DNA. These clones are used as probes to detect viral sequences in cell lines other than H9/HTLV-III, taken from different AIDS patients, and in fresh lymphoid tissues of AIDS patients, providing further evidence that the cloned genomes constitute predominant forms of HTLV-III, the causative agent in AIDS.

Statement of Utility

Previous work with the HTLV family of virus showed three variants. Of these, it was believed that HTLV-III was the causative agent of AIDS. Using the clones produced by this invention, HTLV-III has been shown to be distinctly different than HTLV-I and HTLV-II, whereas HTLV-I and -II share greater homology and thus better identification of AIDS virus in sera.

Description of the Figures

Figure 1 is a Southern blot analysis of unintegrated DNA of HTLV-III. No viral sequences could be detected in the undigested DNA after 4 hours. However, a major species of viral DNA of approximately 10 Kb length was present in the 10, 15, 24 and 48 hr harvest representing the linear unintegrated form of the virus. A representative Southern blot of the 15 hr harvest digested with several restriction enzymes is shown in this figure. Methods: 8 x 10^8 fresh uninfected H9 cells were infected with concentrated supernatant from cell line H9/HTLV-III containing 4 X 10^{11} particles of HTLV-III. Infected cells were divided into five Roller bottles and harvested after 4, 10, 15, 24 and 48 hrs. Low molecular weight DNA was prepared using the Hirt fractionation procedure and 30 ug of undigested and digested DNA were separated on a 0.8% agarose gel, transferred to nitrocellulose paper and hybridized to a HTLV-III cDNA probe for 24 hr at 37°C in 1 X SSC, 40% formamide and 10% Dextran sulfate. cDNA was synthesized from poly(A) selected RNA prepared from doubly banded HTLV-III virus in the presence of oligo(dT) primers. Filters were washed at 1 X SSC at 65°C.

Figure 2 is a restriction endonuclease map of two closely related HTLV-III variants cloned from unintegrated viral DNA. Three recombinant clones (λ BH10, λBH5 and λBH8) were analyzed and their inserts (9 Kb, 5.5 Kb and 3.5 Kb, respectively) were mapped with the indicated enzymes. They represent two variant forms of HTLV-III differing in three enzyme sites which are depicted in bold letters and by an asterisk. As SstI cuts the LTR of the HTLV-III the three clones represent two full length genomes with one LTR. A schematic map of this viral genome is shown at the bottom of the

figure, although the total length of the LTR is approximate. Methods: Low molecular weight DNA combined from the 15 and 24 hr harvest was fractionated on a 10-40% sucrose gradient. Aliquots of the fractions were electrophoresed on a 0.5% agarose gel, transferred to nitrocellulose paper and hybridized to HTLV cDNA under conditions described in Figure 1. Fractions which contained the unintegrated linear HTLV-III genome shown by hybridization were pooled, the DNA was subsequently digested with SstI and ligated to phosphatase treated SstI arms of λgtWes·λB. After in vitro packaging, recombinant phages were screened for viral sequences with HTLV-III cDNA.

Figure 3 demonstrates HTLV-III viral sequences in the infected cell line H9/HTLV-III. Both variant forms of HTLV-III were detected as integrated provirus as well as unintegrated viral DNA in the infected cell line. However, no viral sequences were found in uninfected H9 cells, uninfected HT cells nor in normal human thymus (NT). Methods: 10 µg of high molecular weight DNA were digested with restriction enzymes as indicated and hybridized to nick translated phage insert from BH10 under the same conditions as described in Figure 1.

Figure 4 shows a sequence homology of HTLV-III to other members of the HTLV family. A schematic restriction map of HTLV-I, HTLV-Ib and HTLV-II is drawn below indicating the length and the location of the generated fragments in respect to the corresponding genomic regions. LTR, gag, pol, env and pX regions are drawn to scale according to the published nucleotide sequence of HTLV-I. The bands which are most highly conserved as stringency increases correspond to the gag/pol junction region of HTLV-I (1.8 Kb PstI fragment) and HTLV-IIb (3.1 Kb PstI fragment) and to the 3' part

of the pol region of HTLV-II (2.1 Kb SmaI/BamHI fragment) which do not overlap assuming the same genomic organization in HTLV-II. Fragments corresponding to pX of HTLV-I (2.1 Kb SstI Pst fragment) and HTLV-Ib (1.4 Kb Pst fragment) are less conserved but still visible at Tm - 28°C on the original autoradiogram. Digestion of GaLV generates six fragments, none of which show hybridization under medium or high stringency. Methods: Subclones of full length genomes of a prototype HTLV-I, HTLV-Ib, HTLV-III and GaLV (Seato strain) were digested with the following enzymes, PstI plus SstI (HTLV-I and HTLV-Ib), BamHI plus SmaI (HTLV-II) and Hind III plus SmaI plus XhoI (GaLV). Four replicate filters were prepared and hybridized for 36 hr under low stringency (8 X SSC, 20% formamide, 10% Dextran sulfate at 37°C) to nick translated insert of λ BH10. Filters were then washed in 1 X SSC at different temperatures, 22°C (Tm - 70°C) filter 1, 37°C (Tm - 56°C) filter 2, 50°C (Tm - 42°C) filter 3 and 65°C (Tm - 28°C).

The Invention

The present invention discloses a method for production of molecular clones of HTLV-III from a fraction enriched for the unintegrated provirus in acutely infected cells. Three clones for the HTLV-III genome were produced using recombinant DNA techniques by isolating and characterizing unintegrated viral DNA, cleaving this DNA with the appropriate restriction enzyme, and constructing a phage library capable of being screened by viral cDNA. This process led to the production of three clones: BH10, containing a viral insert of 9.0 Kb corresponding to the complete HTLV-III genome; clone BH8 containing an insert of 5.5 Kb; and clone BH5 containing a viral insert of 3.5 Kb. See Figure 3 for a pictoral representation of the differences between these three clones.

In general, cloning the HTLV-III genome involved isolating unintegrated viral DNA after infection of H9-cells with concentrated HTLV-III virus and cloning this DNA in a lambda phage library to be screened with viral cDNA. The cell line H9/HTLV-III produces large quantities of HTLV-III virus and serves as the principal producer cell line for immunological assays used to detect virus specific antigens and antibodies in AIDS sera. Cultures of H9/HTLV-III cells (infected cells) are grown and harvested, followed by extraction of low molecular weight DNA from the newly infected cells. This produces unintegrated viral DNA. A cDNA library is formed using HTLV-III cDNA. This cDNA is then used as a probe for assaying the unintegrated viral DNA. Unintegrated linear DNA (provirus DNA) is then obtained, containing the entire HTLV-III genome, i.e., replication competent. This DNA is then digested in plasmid lambda gt Wes · lambda B to form clone lambda BH10. The other clones are produced by digesting provirus DNA that does not contain the entire HTLV-III genome.

Two elements of the above process are recombinant DNA procedures, such as, the DNA library and a cDNA probe. The library is formed by taking the total DNA from H9/HTLV-III cells, cutting the DNA into fragments with a suitable restriction enzyme, hybridizing to the fragments to a radiolabeled cDNA probe, joining the fragments to plasmid vectors, and then introducing the recombinant DNA into a suitable host.

The cDNA probe is an HTLV-III cDNA probe made from double-banded HTLV-III mRNA. A short oligo-dT chain is hybridized to the poly-A tail of the mRNA strand. The oligo-T segment serves as a primer for the action of reverse transcriptase, which uses the mRNA as a template for the synthesis of a complementary DNA strand. The resulting cDNA ends in a hairpin loop.

Once the mRNA strand is degraded by treatment with NaOH, the hairpin loop becomes a primer for DNA polymerase I, which completes the paired DNA strand. The loop is then cleaved by S1 nuclease to produce a double-stranded cDNA molecule. Linkers are then added to the double-stranded cDNA by using DNA ligase. After the linkers are cut open with a restriction enzyme and the cDNA is inserted into a suitable plasmid cleaved with the same enzyme, such as pBR322. The result is a cDNA-containing recombinant plasmid.

Statement of Deposit

The cell lines and clones of this invention are on deposit in the American Type Culture Collection in the manner prescribed by the Patent and Trademark Office with regard to permanence of the deposit for the life of the patent and without restriction on public access. The accession numbers are: H9/HTLV-III, CRL 8543; BH10, #40125; BH8, #40127; and BH5, #40126.

Specific Disclosure

Concentrated virus from H9/HTLV-III is used to infect fresh uninfected H9 cells at a multiplicity of 50 viral particles/cell; cultures are harvested after 4, 10, 15, 24 and 48 hours. Extrachromosomal DNA is extracted according to the procedure of Hirt and assayed for its content of unintegrated viral DNA using HTLV-III cDNA as a probe. This cDNA is primed by oligo(dT) and copied from poly(A) containing RNA from virions that had been twice banded on sucrose density gradients. Unintegrated linear viral DNA is first detected after 10 hrs and is also present at the subsequent time points. A Southern blot of the 15 hr harvest is shown in Figure 1. A band of approximately 10 Kb in the undigested DNA represents the linear form of the unintegrated,

replication-competent HTLV-III. No closed or nicked circular DNA could be detected in the 10, 15 and 24 hour harvest, but both forms were evident in small amounts at the 48 hr harvest (data not shown). The viral genome was not cut by XbaI, whereas SstI generated three predominant bands of 9 Kb, 5.5 Kb and 3.5 Kb (Figure 1). These bands represent the complete genomes of two forms of HTLV-III, both cut by SstI in the LTR and one having an additional SstI site in the middle of its genome. Clone BH10 contains a viral insert of 9.0 Kb, a size consistent with the complete HTLV-III genome. Clones BH8 and BH5 contain inserts of 5.5 Kb and 3.5 Kb, respectively, and together they overlap completely with BH10, except for a few restriction enzyme sites polymorphisms in BH5. Therefore, BH10 and BH8 plus BH5 represent two variants of HTLV-III.

EXAMPLE 1

In order to demonstrate the presence of these two variants in the original cell line, nick-translated inserts of lambda BH10 was hybridized to a Southern blot of H9/HTLV-III genomic cDNA digested with several restriction enzymes (Figure 3). Both forms could be detected using the enzyme SstI generating the expected 3 bands of 9.0 Kb, 5.5 Kb and 3.5 Kb Xba which does not cut the provirus generating a high molecular weight genome representing polyclonal integration of the provirus and a band of approximately 10 Kb which could be interpreted as representing unintegrated viral DNA since a band of identical size was also present in the undigested first preparation (Figure 1). This was confirmed by Southern blot hybridization of undigested cellular DNA. The existence of unintegrated viral DNA thus explains the presence of a 4 Kb and 4.5 Kb EcoRI fragment seen in both first and total cellular DNA preparations (Figure 1 and Figure 3). BglII and HindIII

both cut the LTR and generated the expected internal bands. Several faint bands in the HindIII digest, in addition to the internal bands, represent either defective proviruses or another variant form with differences in the HindIII restriction pattern. The lack of HTLV-III sequences in the uninfected H9 cell line and the uninfected parental line HT as well as in normal human thymus demonstrated the exogenous nature of HTLV-III and showed that the virus does not contain any human cellular sequences. The same results were obtained using nick-translated inserts from lambda BH5 and lambda BH8.

EXAMPLE 2

The availability of the cloned HTLV-III genome allowed sequence homology between HTLV-III, HTLV-I and HTLV-II to be evaluated. Replicate Southern blots of restriction enzyme digested clones representing the complete genomes of HTLV-I, HTLV-Ib, HTLV-II and GALV as a control were hybridized to full length HTLV-III probe under relaxed conditions. The filters were then washed (λ BH10i) under conditions of low, medium, and high stringencies in order to estimate the extent of homology between HTLV-III and these viruses (Figure 4). This experiment showed that there is specific homology between HTLV-III, HTLV-I, HTLV-Ib and HTLV-II but not with HTLV-III and GALV. As demonstrated, hybridization of HTLV-III to other members of the HTLV family could be detected at the values of -42°C and -28°C, conditions under which no hybridization to GALV was seen (Figure 4, panels C and D). Of note, the restriction fragments showing greatest homology correspond to the gag/pol region of HTLV-I and to an apparently non-overlapping portion of the pol region of HTLV-II (assuming that the genomic arrangement is similar to that of HTLV-I). Further analysis revealed that it is the 5' half of the

gag and the gap between gag and pol which has the greatest homology in HTLV-I. Finally, in HTLV-Ib (a variant of HTLV-I) hybridization to the px region could be seen (1.4 Kb Pst fragment) as well as to the corresponding px fragment in HTLV-I (2.1 Kb Pst/Sst) on the original autoradiogram.

EXAMPLE 3

Figure 2 shows the restriction map of three clones designated λBH10, λBH5 and λBH8 which correspond in size to the three SstI fragments shown in Figure 1. Comparison of these maps suggests that λBH5 plus λBH8 constitute one HTLV-III genome, and λBH10 another. The two viral forms differ in only three out of 21 mapped enzyme sites, including the internal SstI site. As expected, the phage inserts of λBH5 and λBH8 hybridize under high stringency conditions to λBH10 but not to each other as analyzed by Southern blot hybridization and electron microscopic heteroduplex analysis. To show the presence of LTR sequences in the clones and to determine their orientation, a cDNA clone (C15) was used as a probe and contained U3 and R sequences. This clone strongly hybridized to the 0.5 Kb BglII fragment of λBH10 and λBH8, orienting this side 3', and faintly hybridized to the 0.7 Kb SstI/PstI fragment of λBH5 and λBH10, orienting this side 5', and demonstrated that SstI cuts the LTR of HTLV-III in the R region.

EXAMPLE 4

The presence of two variant forms of HTLV-III in the original cell line was demonstrated by hybridizing the radiolabelled insert of λBH10 to a Southern blot of H9/HTLV-III genomic DNA digested with several restriction enzymes (Figure 3). Both forms were detected using the enzyme SstI which generated the

expected 3 bands of 9Kb, 5.5 Kb and 3.5 Kb length. Both of these forms are also present as integrated proviruses because they have been cloned along with their flanking cellular sequences from a genomic library of H9/HTLV-III. Furthermore, XbaI, which does not cut the provirus, generated a high molecular weight smear representing polyclonal integration of the provirus and a band of approximately 10 Kb, representing unintegrated viral DNA. This same 10 Kb band was also detected in undigested H9/HTLV-III DNA, again indicating unintegrated viral DNA. The presence of unintegrated viral DNA also explains the 4 Kb and 4.5 Kb EcoRI fragment seen in both the Hirt and total cellular DNA preparations (Figures 1, 3). Bgl II and Hind III both cut the LTR and generate the expected internal bands. Several faint bands, in addition to the internal bands using Hind III, represent either defective proviruses or another variant form present in low copy number. The lack of HTLV-III sequences in the DNA of the uninfected H9 cell line and the uninfected parental cell line HT as well as in normal human thymus clearly demonstrates the exogenous nature of HTLV-III and shows that the virus does not contain human cellular sequences. The same results were obtained using λBH5 and λBH8 as probe inserts.

WE CLAIM

1. Recombinant clone BH10 characterized by containing the complete HTLV-III genome.

2. Recombinant clone BH8 characterized by containing a 5.5 Kb viral insert from HTLV-III virus.

3. Recombinant clone BH5 characterized by containing a 3.5 Kb viral insert from HTLV-III.

4. A process for the production of recombinant molecular clones of HTLV-III consisting essentially of cleaving unintegrated viral DNA from HTLV-III cells with a restriction enzyme to obtain a provirus, hybridizing radiolabeled cDNA to said provirus, and digesting said virus in a suitable plasmid.

5. A process for the molecular cloning and expression of a cDNA sequence of HTLV-III consisting essentially of

isolating total cellular mRNA from H9/HTLV-III cells;

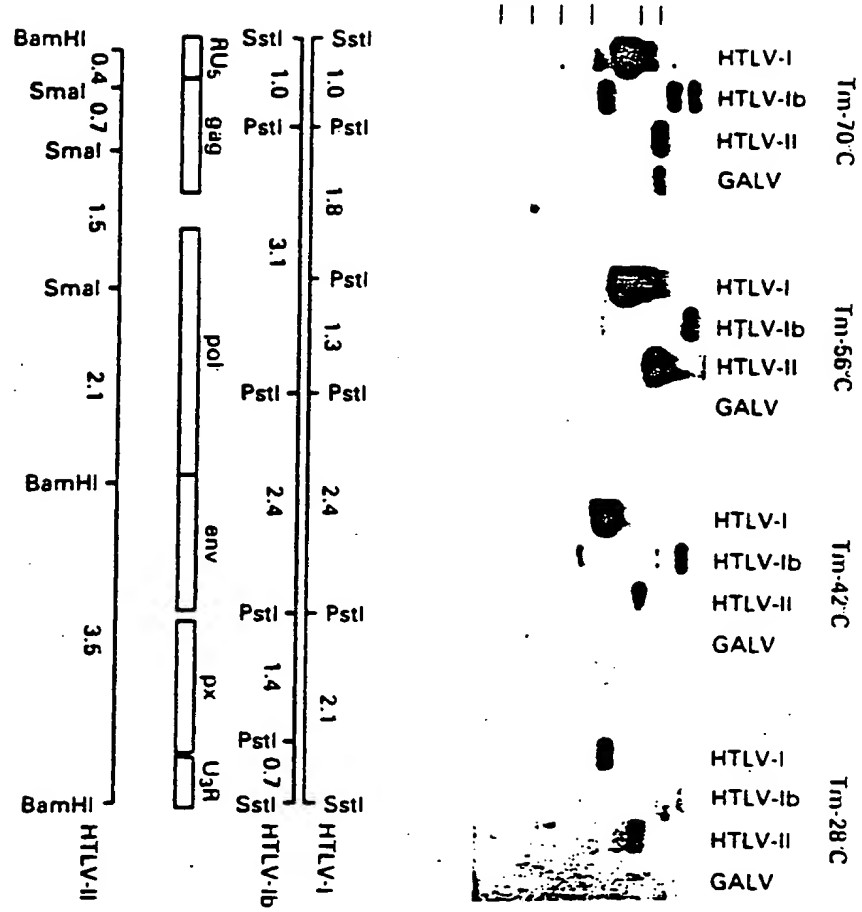
forming double-stranded cDNA from said mRNA and inserting said double-stranded cDNA into a phage lambda to form a recombinant DNA molecule;

hybridizing said recombinant DNA molecule with a radiolabelled probe;

removing cDNA from said molecules and inserting said cDNA into a suitable plasmid; and

transfecting said plasmids into a suitable host cell capable of expressing HTLV-III DNA sequences.

6. A process of Claim 5 wherein said plasmid is λBH10.



F16 #4 JWS 8/17/84
8/18/84
RG/ 8/17/84
MPJ 8/17/84

FIG 3 DWS
8/17/84

RC
8/17/84
8/17/84
MP/8/17/84

HTLV-III a
L1
Sst I
Hind III
Pst I
Hind III
Bgl II
Kpn I
Kpn I
EcoR I
EcoR I
Sal I
Kpn I
Bgl II
Hind III
BamH I
Xho I
Kpn I
Bgl II
Bgl II
Sst I
R1
IBH · 10
HTLV-III b
L1
Sst I
Hind III
Pst I
Hind III
Bgl II
Kpn I
Kpn I
EcoR I
EccR I
Sal I
*Sst I
R1
IBH · 5
L1
Sst I*
Kpn I
Bgl II
Hind III
BamH I
Hpa I*
Xho I
Kpn I
Bgl II
BamH I*
Bgl II
Sst I
R1
IBH · 8
5'
RU5
U3R
3'
0
1
2
3
4
5
6
7
8
9
Kb

FIG 2

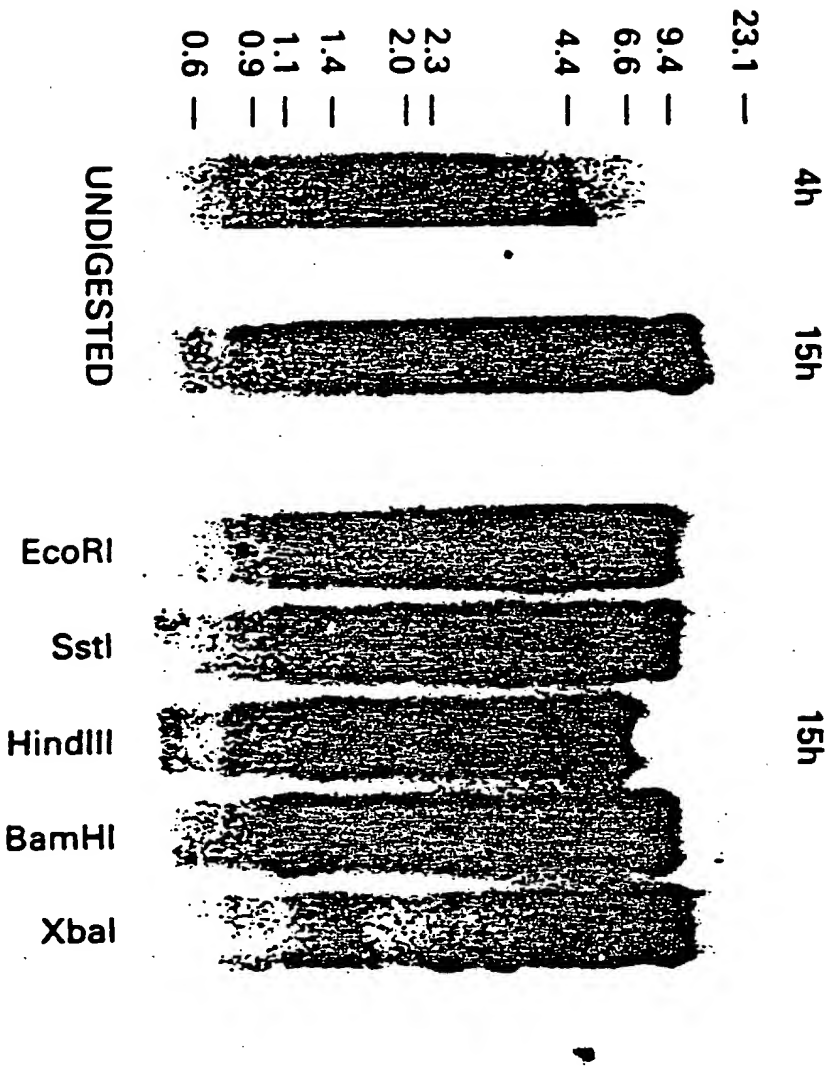

23.1
9.4
6.6
4.4
2.3
2.0
1.4
1.1
0.9
0.6
4h
15h
UNDIGESTED
EcoRI
SstI
HindIII
BamHI
XbaI
15h

FIG 1

7. A process of Claim 5 wherein said plasmid is λ BH8.

8. A process of Claim 5 wherein said plasmid is λ BH5.

9. A process of Claim 5 wherein said cDNA sequence corresponds to a 9.0 Kb sequence.

10. A process of Claim 5 wherein said cDNA sequence corresponds to a 5.5 Kb sequence.

11. A process of Claim 5 wherein said cDNA sequence corresponds to a 3.5 Kb sequence.

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

October 31, 1995

THIS IS TO CERTIFY THAT ANNEXED IS A TRUE COPY FROM THE RECORDS OF THIS OFFICE OF THE FILE WRAPPER AND CONTENTS OF:

APPLICATION NUMBER: *06/659,339*
FILING DATE: *October 10, 1984*
TITLE OF INVENTION:
CLONING AND EXPRESSION OF HTLV-III DNA
INVENTOR(S):
CHARLES F. MCNEELY T.

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

Sandra Allen

SANDRA ALLEN
Certifying Officer

REGULAR UTILIT[redacted]

Form PTO-436
(Rev 8/78)

APPLICATION H. B...COME ABANDONED. THIS NOTICE MAILED:

SERIAL NUMBER (Series of 1979)	PATENT DATE	PATENT NUMBER
C59339		

SERIAL NUMBER	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER
C6/659,339	10/10/84	436 - 35		~~320~~ 127	[signature]

APPLICANTS: NANCY T. CHANG, PAOLI, PA.

CONTINUING DATA***********************
VERIFIED

FOREIGN/PCT APPLICATIONS***********
VERIFIED

FOREIGN FILING LICENSE GRANTED 01/28/85 ***** SMALL ENTITY *****

Foreign priority claimed ☐ yes ☐ no / 35 USC 119 conditions met ☐ yes ☐ no / Verified and Acknowledged — Examiner's Initials	AS FILED →	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
		PA	6	40	22	$ 585.00	CT884-7

ADDRESS:
DAVID E. BROOK
HAMILTON, BROOK, SMITH & REYNOLDS
2 MILITIA DR.
LEXINGTON, MA [redacted]173

TITLE: CLONING AND EXPRESSION OF HTLV-III DNA

U.S. DEPT. of COMM.-Pat. & TM Office — PTO-436L (rev. 10-78)

PARTS OF APPLICATION FILED SEPARATELY

PREPARED FOR ISSUE

CTRB
DEB:gm:jzg
10/10/84

OCT 21 1984 PAT. & TRADE MARK OFF.

-1-

200.00 - 1
570.00 - 1c

CC59339

a

CLONING AND EXPRESSION OF HTLV-III DNA

Description

Technical Fields

This invention is in the fields of biology and virology and in particular relates to human T cell leukemia virus - type III (HTLV-III).

Background Art

The term human T cell leukemia-lymphoma virus (HTLV) refers to a unique family of T cell tropic retroviruses. Such viruses play an important role in the pathogenesis of certain T cell neoplasms. There are presently three known types of HTLVs. One subgroup of the family, HTLV-type I (HTLV-I) is linked to the cause of adult T-cell leukemia-lymphoma (ATLL) that occurs in certain regions of Japan, the Caribbean and Africa. HTLV-type II (HTLV-II) has been isolated from a patient with a T-cell variant of hairy cell leukemia. M. Popovic et al., Detection, Isolation, and Continuous Production of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and Pre-AIDS, Science, 224:497-

12/07/84 659339 1 201 ~~300.00~~ CK
12/07/84 659339 1 203 200.00 CK
12/07/84 659339 1 202 570.00 CK

HTLV-type III (HTLV-III) has been isolated from many patients with acquired immune deficiency syndrome (AIDS). It refers to prototype virus isolated from AIDS patients. Groups reported to be at greatest risk for AIDS include homosexual or bisexual males; intravenous drug users and Haitian immigrants to the United States. ~~homosexual~~ or ~~bisexual males~~ having ~~heterosexual~~ contact ~~with~~

S2062 12/06/84 659339 08-0380 1 201 150.00CR
S2063 12/06/84 659339 08-0380 1 203 100.00CR
S2064 12/06/84 659339 08-0380 1 202 285.00CR

~~12/07/84 659339 1 101 300.00 CK~~
~~12/07/84 659339 1 103 200.00 CK~~
~~12/07/84 659339 1 102 570.00 CK~~

hemophiliacs who receive blood products pooled from donors and recipients of multiple blood transfusions are also at risk. Clinical manifestations include severe, unexplained immune deficiency which generally involves a depletion of helper T lymphocytes. These may be accompanied by malignancies and infections. The mortality rate for those with AIDS is high. A less severe form of AIDS also exists, in which there may be lymphadenopathy and depressed helper T cell counts; there is not, however, the devasting illness characteristic of full-blown AIDS. There are many individuals, who are classified as having early AIDS (pre-AIDS), who exhibit these signs. It is not now possible to predict who among them will develop the more serious symptoms.

Much of the evidence implicates HTLV-III as the etiological agent of the infectious AIDS. First, there is consistent epidemiology; greater than 95% of the patients with AIDS have antibodies specific for HTLV-III. Second, there has been reproducible identification and isolation of virus in this disease; more than 100 variants of HTLV-III have been isolated from AIDS patients. Third, there has been transmission of the disease to normal healthy individuals who received blood transfusions from infected blood donors.

HTLV-III has been shown to share several properties with HTLV-I and HTLV-II but also to be morphologically, biologically and antigenically distinguishable. R.C. Gallo et al., Frequent Detection and Isolation of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and At Risk for AIDS. Science, 224:500-503. (1984). For example,

HTLV-III has been shown to be antigenically related to HTLV-I and HTLV-II by demonstrating cross-reactivity with antibodies to HTLV-I and HTLV-II core proteins, P24 and P19, and envelope antigens and by nucleic acid cross-hybridization studies with cloned HTLV-I and HTLV-II DNAs. However, unlike HTLV-I and HTLV-II, it lacked the ability to infect and transform T cells from normal umbilical cord blood and bone marrow in vitro, and has the cytopathic effect on infected cells only.

Like the RNA genome of other retroviruses, the RNA genome of HTLV-III contains three genes which encode viral proteins: 1) the gag gene, which encodes the internal structural (nucleocapsid or core) proteins; 2) the pol gene, which encodes the RNA-directed DNA polymerase (reverse transcriptase); and 3) the env gene, which encodes the envelope glycoproteins of the virion. In addition, the HTLV-III genome contains a region designated Px, located between the env gene and the 3' LTR, which appears to be involved in functional killing of the virus.

At this time, AIDS is still difficult to diagnose before the onset of clinical manifestations and impossible to treat or even prevent.

Summary of the Invention

This invention is based upon applicant's cloning of HTLV-III DNA in recombinant/vector host systems capable of expressing immunoreactive HTLV-III polypeptides. In one embodiment, an immunoreactive protein coded for by an env gene sequence of HTLV-III has been produced by these recombinant

DNA methods. This polypeptide is immunoreactive with sera of patients having acquired immunodeficiency syndrome or antibodies to HTLV-III. The polypeptide expressed has been isolated.

In another embodiment of the invention, immunoreactive polypeptides produced by the recombinant DNA methods are employed in the production of antibodies, including monoclonal antibodies, reactive with the polypeptides. Such antibodies form the basis for immunoassay and diagnostice techniques for detecting HTLV-III, particularly in body fluids such as blood, saliva, urine, etc.

In another embodiment of the invention, DNA probes are formed from DNA sequences coding for portions of the HTLV-III genome. Such DNA probes can also be employed in detecting the presence of HTLV-III in blood or other fluids.

Diagnostic kits including immunoreactive polypeptides, DNA probes, etc. can also be produced to include any of the products of this invention.

Brief Description of the Figures

Figure 1 is a representation of HTLV-III DNA. Figure 1a shows sites at which the genome is cut by the restriction enzyme SstI and Figure 1b shows the fragments of HTLV-III genome produced through the action of restriction enzymes Kpn, EcoRI and Hind III.

Figure 2 is a representation of HTLV-III DNA and the location of restriction enzyme sites in the genome.

Figure 3 shows nucleotide sequences for HTLV-III DNA which encompasses the env region.

Figure 4 is an immunoblot showing the position on an SDS polyacrylamide gel of HTLV-III env-Beta-galactosidase fusion proteins.

Best Mode of Carrying Out the Invention

The envelope glycoprotein is the major antigen recognized by the antiserum of AIDS patients. In this respect, HTLV resembles other retroviruses, for which the envelope glycoprotein is typically the most antigenic viral polypeptide. In addition, the neutralizing antibodies are generally directed toward the envelope glycoprotein of the retrovirus. Serum samples from 88 percent to 100 percent of those with AIDS have been shown to have antibodies reactive with antigens of HTLV-III; the major immune reactivity was directed against p41, the presumed envelope antigen of HTLV-III. Antibodies to core proteins have also been demonstrated in serum of AIDS patients, but are evidently not as effective an indicator of infection as is the presence of antibodies to envelope antigen.

The p41 antigen of HTLV-III has been difficult to characterize because the viral envelope is partially destroyed during the process of virus inactivation and purification. The present invention responds to the great need to characterize the antigenic component of the HTLV-III virus--and thus provide screening, diagnostic and preventive products and methods--in several ways.

First, the present invention relates to the isolation of genes of HTLV-III which encode

immunoreactive polypeptides; identification of the nucleotide sequence of these genes; introduction of DNA sequences specific to these viral DNA sequences into appropriate vectors to produce viral RNA and the formation of DNA probes. These probes are comprised of sequences specific to HTLV-III DNA and are useful, for example, for detecting the same HTLV-III DNA sequences in body fluids (e.g., blood).

Second, the present invention relates to HTLV-III polypeptides which are produced by translation of the recombinant DNA sequences encoding HTLV-III proteins. Polypeptides which are so produced and which are immunoreactive with serum from AIDS patients are referred to as recombinant DNA-produced immunoreactive HTLV-III polypeptides. They include, but are not limited to, antigenic HTLV-III core and envelope polypeptides which are produced by translation of the recombinant DNA sequences specific to the gag and the env DNA sequences encoding HTLV-III core proteins and envelope glycoproteins, respectively. They also include the polypeptides which are produced by translation of the recombinant DNA sequences specific to the Px genes of HTLV-III. The polypeptides may be used as vaccines for the prevention of AIDS. The methods of producing the polypeptides are also a subject of this invention, as are diagnostic methods based on these polypeptides.

Third, the present invention also relates to antibodies against the immunoreactive HTLV-III polypeptides which are the subject of this invention. These antibodies are the basis for assays

relating to the diagnosis of AIDS or the presence of HTLV-III in body fluids.

In one embodiment of this invention, genetic engineering methods are used to isolate DNA sequences of HTLV-III which encode immunoreactive HTLV-III polypeptides, such as the core protein and the envelope glycoprotein, and to identify the nucleotides which comprise those sequences. The proviral genes integrated into host cell DNA are molecularly cloned and the nucleotide sequences of the cloned provirus is determined.

An E. coli expression library of HTLV-III DNA is constructed; in this library are vectors harboring HTLV-III DNA sequences. The HTLV-III genome is cloned and cuts are then made in the cloned HTLV-III genome with restriction enzymes to produce DNA fragments. (Figures 1 and 2) HTLV-III DNA fragments of approximately 200-500bp are isolated from agarose gel, end repaired with T_4 polymerase and ligated to linker DNA. The linker ligated DNA is then treated with a restriction enzyme, purified from agarose gel and cloned in an expression vector. Examples of the expression vectors used are: OmpA, pIN (A,B and C), lambda pL, T7, lac, Trp, ORF and lambda gt11. In addition, mammalian cell vectors such as pSV28pt, pSV2neo, pSVdhfr and VPV vectors, and yeast vectors, such as GALI and GAL10, may be used.

The bacterial vectors contain the lac coding sequences, into which HTLV-III DNA can be inserted for the generation of B-galactosidase fusion protein. The hybrid molecules are then introduced into bacteria (e.g., E.coli); those cells which take up a

vector containing HTLV-III DNA are said to be transformed. The bacteria are plated on top of MacConkey agar plates in order to verify the phenotype of clone. If functional B-galactosidase is being produced, the colony will appear red.

Bacterial colonies are also screened with HTLV-III DNA probes containing the DNA regions of interest (e.g., HTLV-III gag and env DNA sequences). This results in identification of those clones containing the insert. Clones which are positive when screened with the DNA probe and positive on the MacConkey agar plates are isolated.

This identification of cells harboring the HTLV-III DNA sequences makes it possible to produce HTLV-III polypeptides which are immunoreactive with HTLV-III specific antibody. The cells from the selected colonies are grown in culture under conditions conducive to allowing the expression of the hybrid protein. The culture is spun down and the resulting cell pellet broken. The total cellular protein is analysed by being run on an SDS polyacrylamide gel. The fusion proteins are identified at a position on the gel which contains no other protein. (Figure 2) Western blot analyses are also carried out on the clones which screened positive. Such analyses are carried out using serum from AIDS patients, with the result that it is possible to identify those clones expressing HTLV-III env-B-galactosidase fusion proteins (antigens) that cross-react with the HTLV-III specific antibody.

In another embodiment of this invention, lambda $_{10}$ clones harboring HTLV-III DNA are cloned

from the replicated form of the virus. As the retrovirus is replicating, double stranded DNA is being produced. Cuts are made in the cloned HTLV-III DNA with the restriction enzyme SstI. (Figure 1a) Because there are two SstI recognition sites within the LTR of HTLV-III DNA, one LTR region is not present in the cloned DNA sequence removed from the $lambda_{10}$ vector. As a result, a small (approximately 200 bp) fragment of the HTLV-III DNA is missing.

The resulting DNA is linearized and fragments are produced by digesting the linearized genomic DNA spanning the env gene region with restriction enzymes. For example, fragments are produced using Kpn or EcoRI plus HindIII, as shown in Figure 1b. The resulting 2.3kb KpnI-KpnI fragments; 1.0kbEcoRI-EcoRI fragments and 2.4Kb EcoRI-HindIII fragments are isolated by gel electrophoresis and electro-elution. These fragments are randomly sheared to produce fragments. The fragments thus produced are purified from agarose gel and DNA fragments between about 200-500 bp are eluted.

The eluted 200-500bp DNA fragments are end filled through the use of E. coli T_4 polymerase and blunt end ligated into an open reading frame expression (ORF) vector, such as pMR100. This ligation may occur at the SmaI site of the pMR100 vector, which contains two promoter regions, hybrid coding sequences of lambdaCI gene and lacI-LacZ gene fusion sequence. In the vector, these are out of frame sequences; as a result, the vector is nonproductive. The HTLV-III DNA is inserted into the vector; the correct DNA fragments will correct the reading

frame, with the result that CI-HTLV-III-B-galactosidase fusion proteins are produced. The expression of the hybrid is under the control of the lac promoter.

Based on the sequence of pMR100, it appears that if a DNA fragment insert cloned into the SmaI site is to generate a proper open reading frame between the lambdaCI gene fragment and the lac-7 fragment, the inserted DNA must not contain any stop codons in the reading frame set by the frame of the lambdaCI gene.

The hybrid molecules are then introduced into E. coli. The bacteria are plated on MacConkey agar plates to verify the phenotype of the clone. If functional B-galactosidase is being produced, the colony will appear red. The colonies are also screened with HTLV-III DNA probes, for the purpose of identifying those clones containing the insert. Clones which are positive when screened with the DNA probe and positive on the MacConkey agar plates are isolated.

The cells from the selected colonies are grown in culture. The culture is spun down and the cell pellet broken. Total cellular protein is analysed by being run on an SDS polyacrylamide gel. The fusion proteins are identified at a position on the gel which contains no other protein. (Figure 4)

Western blot analyses are also carried out on the clones which screened positive. Sera from AIDS patients are used, thus making it possible to identify those clones which express the HTLV-III-env-B-galactosidase fusion proteins (antigens) that cross-react with the HTLV-III specific antibody.

1000 clones were screened by this method; 6 were positive.

Because of the nature of the pMR100 cloning vehicle, a productive DNA insert should also be expressed as a part of a larger fusion polypeptide. HTLV-III env gene containing recombinant clones was identified by colony hybridization. The production of larger fusion polypeptides bearing functional B-galactosidase activity was verified by phenotype identification on MacConkey agar plates; by B-galactosidase enzyamatic assays and by analysis on 7.5% SDS-polyacrylamide gels. Immunoreactivity of the larger protein with antibody to HTLV-III was assessed by western blot analysis using serum from AIDS patients. These large fusion proteins also reacted with anti-B-galactosidase and anti-CI antiserum. This finding is consistent with the hypothesis that they are proteins of CI-HTLV-III-lacIZ.

The open reading frame insert fragment of HTLV-III is further analyzed by DNA sequencing analysis. Because one of the two BamHI sites flanking the SmaI cloning site in pMR100 is destroyed in the cloning step, positive clones are digested with restriction enzymes HindIII and claI to liberate the inserted HTLV-III DNA fragment. The HTLV-III ORF inserts are isolated from the fusion recombinant and cloned into M13 sequencing cloning vector mp18 and mp19 digested with HindIII and AccI. DNA sequences of the positive ORF clones are then determined.

In another embodiment of this invention, fragments of HTLV-III DNA of approximately 200-500

bps are isolated from agarose gel, end repaired with T_4 polymerase and ligated to EcoRI linker. The EcoRI linker ligated DNA is then treated with EcoRI purified from 1% agarose gel and cloned in an expression vector, gt11. This vector contains lac Z gene coding sequences into which the foreign DNA can be inserted for the generation of B-galactosidase fusion protein. The expression of the hybrid gene is under the control of lac repressor. The lac repressor gene, lac I, is carried on a separate plasmid pMC9 in the host cell, E. coli Y1090. AIDS patient serum was used to probe the gt11 library of HTLV-III genome DNA containing 1.5×10^4 recombinant phage. In a screen of 5000 recombinants, 100 independent clones that produced strong signals were isolated. The positive recombinant DNA clones were further characterized for their specific gene expression. Rabbit hyperimmune serum against P24 was also used to identify the gag gene specific clones. Nick-translated DNA probes of specific HTLV-III gene, specifically the gag gene, env gene and Px gene were used to group the positive immunoreactive clones into specific gene region.

Recombinant clones that produced strong signals with AIDS serum and contain insert DNA spanning the HTLV-III env gene region were examined in detail by mapping their insert with restriction enzymes and DNA sequencing analysis.

Another embodiment of this invention relates to the formation of RNA and RNA probes specific to the HTLV-III DNA of this invention. DNA sequences which are an entire gene or segment of a gene from HTLV-III are inserted into a vector, such as a T7 vector.

In this embodiment, the vector has the Tceu promoter from the T cell gene 10 promoter and eleven amino acids from the T cell gene 10 protein.

The vectors are then used to transform cells, such as E. coli. The T7 vector makes use of the T7 polymerase, which catalyzes RNA formation and recognizes only T7 promoter, which is the site where RNA polymerase binds for the initiation of transcription. This vector does not, however, recognize E. coli promoter. As a result, if HTLV-III DNA sequences are inserted after the promoter and polymerase genes of the T7 vector, which recognizes them to the exclusion of other signals, and a terminator is placed immediately after the HTLV-III DNA sequences, the T7 vector will direct manufacture RNA complementary to the HTLV-III DNA insert.

Monoclonal antibodies reactive with HTLV-III envelope polypeptide are produced by antibody-producing cell lines. The antibody-producing cell lines may be hybridoma cell lines commonly known as hybridomas. The hybrid cellsa re formed from the fusion of cells which produce antibody to HTLV-III envelope polypeptide and an immortalizing cell line, that is, a cell line which imparts long term tissue culture stability on the hybrid cell. In the formation of the hybrid cell lines, the first fusion partner - the antibody-producing cell - may be a spleen cell of an animal immunized against HTLV-III envelope polypeptide. Alternatively, the antibody-producing cell may be an anti-HTLV-III envelope polypeptide lymphocyte obtained from the spleen, peripheral blood, lymph nodes or other tissue. The

second fusion partner - the immortal cell - may be a lymphoblastoid cell or a plasmacytoma cell such as a myeloma cell, itself an antibody-producing cell but also malignant.

Murine hybridomas which produce monoclonal antibodies against HTLV-III envelope polypeptide are formed by the fusion of mouse myeloma cells and spleen cells from mice immunized against the polypeptide. To immunize the mice, a variety of different immunization protocols may be followed. For instance mice may receive primary and boosting immunizations of the purified polypeptide. The fusions are accomplished by standard procedures. Kohler and Milstein, (1975) Nature (London) 256, 495-497; Kennet, R., (1980) in Monoclonal Antibodies (Kennet et al., Eds. pp. 365-367, Plenum Press, NY).

The hybridomas are then screened for production of antibody reactive with envelope polypeptide.

Another way of forming the antibody-producing cell line is by transformation of antibody-producing cells. For example, a B lymphocyte obtained from an animal immunized against HTLV-III envelope polypeptide may be infected and transformeed with a virus such as the Epstein-Barr virus in the case of human B lymphocytes to give an immortal antibody-producing cell. See, e.g., Kozbor and Rodor.(1983) Immunology Today 4(3), 72-79. Alternatively, the B lymphocyte may be transformed by a transforming gene or transforming gene product.

The monoclonal antibodies against HTLV-III envelope polypeptide are produced in large quantities by injecting antibody-producing hybridomas into the peritoneal cavity of mice and, after an

appropriate time, harvesting the ascites fluid which contains very high titer of homogenous antibody and isolating the monoclonal antibodies therefrom. Xenogeneic hybridomas should be injected into irradiated or athymic nude mice. Alternatively, the antibodies may be produced by culturing cells which produce HTLV-III envelope polypeptide in vitro and isolating secreted monoclonal antibodies from the cell culture medium.

This invention will now be further illustrated by the following examples. They are not intended to be limiting in any way.

EXAMPLE 1
PREPARATION OF SONICATED DNA FRAGMENTS

10 ug of gel purified HTLV-III restriction fragments were sonicated to fragment size on average of 500 bps. After sonication, the DNA was passed through a DEAE-cellulose column in 0.1XTBE in order to reduce the volume. The DEAE-bound DNA was washed with 5 ml of 0.2 M NaCl-TE (2 M NaCl, 10 mm Tris HCl pH 7.5, 1 mM EDTA) and then eluted with 1 M NaCl-TE, and ethanol precipitated. The size range of the sonicated DNA was then determined on 1.2% agarose gel. DNA fragments of desired length (200-500 bps) was eluted from the gel. T4 DNA polymerase was used to fill in and/or trim the single strand DNA termini generated by the sonication procedure. DNA fragments were incubated with T4 polymerase in the absence of added nucleotides for five minutes at 37°C to remove nucleotides from 3' end and then all 4 nucleotide precursors were added to a final

concentration of 100 uM and the reaction mixture was incubated another 30 minutes to repair the 5'-end single stranded overhang. The reaction was stopped by heat inactivation of the enzyme at 68°C for 10 minutes. DNA was phenol extracted once, ethanol precipitated and resuspended in TE.

EXAMPLE 2

CLONING OF RANDOM SHEARED DNA FRAGMENTS

The sonicated blunt end repaired HTLV-III DNA fragments were ligated into the SmaI site of the ORF expression vector pMR100 and transformed into host cell LG90 using standard transformation procedures. B-galactosidase positive phenotype of the transformant were identified by plating the transformed cell on ampicillin (25 ug/ml) containing McConkey agar plates and scoring the phenotype after 20 hours at 37°C.

EXAMPLE 3

HYBRID PROTEIN ANALYSIS

Ten milliliter samples of cells from an overnight saturated culture grown in L broth containing ampicillin (25 ug/ml) were centrifuged, the cell pellet was resuspended in 500 ul of 1.2 fold concentrated Laemmli sample buffer. The cells were resuspended by vortexing and boiling for 3 minutes at 100°C. The lysate was then repeated by being forced through a 22 guage needle to reduce the lysate viscosity. Approximately 10 ul of the protein samples were electrophoresed in 7.5% SDS-PAGE (SDS-polyacrylamide) gels.

Electrophoretic transfer of proteins from SDS-PAGE gels to nitrocellulose paper was carried out according to Towbin et. al.. After the transfer, the filter was incubated at 37°C for two hours in a solution of 5% (w/v) nonfat milk in PBS containing 0.1% antifoam A and 0.0001% merthiolate to saturate all available protein binding sites. Reactions with AIDS antisera were carried out in the same milk buffer containing 1% AIDS patient antisera that had been preabsorbed with E. coli lysate. Reactions were performed in a sealed plastic bag at 4°C for 18-24 hours on a rotatory shaker. Following this incubation, the filter was washed three times for 20 minutes each at room temperature in a solution containing 0.5% deoxycholic, 0.1 M NaCl, 0.5% triton X-100, 10 mm phosphate buffer pH 7.5 and 0.1 mM PMSF.

To visualize antigen-antibody interactions, the nitrocellulose was then incubated with the second goat antihuman antibody that had been iodinated with ^{125}I. The reaction with the iodinated antibody was carried out at room temperature for 30 minutes in the same milk buffer as was used for the first antibody. The nitrocellulose was then washed as previously described and exposed at -70°C using Kodak XAR5 film with an intensifying screen.

EXAMPLE 4

SCREENING OF THE HTLV-III ORF LIBRARY BY COLONY HYBRIDIZATION

E. coli LG90 transformants were screened with HTLV-III DNA probes containing the DNA regions of interest (e.g. HTLV-III gag, env or Px gene specific

sequences). Colonies were grown on nitrocellulose filter and screened according to the procedure of Grunstein and Hogness by using a nick-translated HTLV-III DNA as hybridization probe.

The DNA fragment was in general exercise by restriction endonuclease digestion, gel purified, and ^{32}P-labeled to a specific activity of 0.5×10^8 cpm/ug by nick-translation (Rigby, P.W.J. et al., J. Mol. Biol. 113, 237 (1977). Duplicate nitrocellulose filters with DNA fixed to them were prehybridized with 6xSSC (0.9 M NaCl/0.09 M sodium citrate, pH 7.0), 5X Denhardt's solution (Denhardt's solution: 0.02% each of polyvinylpyrrolidone, Ficoll and bovine serum albumin) 10 ug of denatured sonicated E. coli DNA per ml at 55°C for 3-5 hours. The filters were then placed in a fresh sample of the same solution to which the denatured hybridization probe had been added. Hybridization was permitted to take place at 68°C for 16 hours. The filters were washed repeatedly in 0.3XSSC at 55°C, and then exposed to x-ray film.

Industrial Applicability

This invention has industrial applicability in screening for the presence of HTLV-III DNA in body fluids and the diagnosis of AIDS.

Equivalents

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substantces and procedures described herein. Such equivalents are considered to be within the

scope of this invention and are covered by the following claims.

CLAIMS

1. Immunoreactive HTLV-III polypeptide expressed by cells transformed with a recombinant vector containing HTLV-III cDNA.

2. A polypeptide of Claim 1 wherein said HTLV-III cDNA encodes an *env* gene sequence.

3. A polypeptide of Claim 2 wherein which is immunoreactive with sera of patients with acquired immunodeficiency syndrome.

4. Isolated HTLV-III envelope polypeptide.

5. Isolated cDNA encoding an HTLV-III gene.

6. cDNA of Claim 5 encoding the HTLV-III *env* gene.

7. Isolated cDNA encoding for an HTLV-III polypeptide which is immunoreactive.

8. Isolated cDNA of Claim 7 coding for an envelope polypeptide which is immunoreactive.

9. A DNA probe comprising a DNA sequence coding a portion of the HTLV-III genome.

10. A DNA probe of Claim 9 wherein the DNA sequence encodes at least a portion of the *env* gene.

11. A hybrid protein comprising an HTLV-III polypeptide linked to at least one other polypeptide.

12. A hybrid protein of Claim 11 comprising an HTLV-III polypeptide linked to an indicator polypeptide.

13. A hybrid protein of Claim 12 wherein said indicator polypeptide comprises beta-galactosidase.

14. An isolated RNA transcript of the env gene of HTLV-III.

15. An isolated RNA transcript of Claim 14 having a label which emits a detectable signal.

16. An isolated RNA transcript of Claim 15 wherein said label comprises a radioisotope.

17. A recombinant vector containing HTLV-III DNA capable of expression upon insertion into host cells.

18. [redacted]pA vector containing HTLV-III cDNA.

19. p[redacted]100 vector containing HTLV-III cDNA.

20. A method of producing HTLV-III polypeptide, comprising the steps of:

a. cleaving HTLV-III cDNA to produce DNA fragments;

b. inserting the DNA fragments into an expression vector to form a recombinant vector;

c. transforming an appropriate host cell with the recombinant vector; and

d. culturing the transformed host cell under conditions sufficient for expression of the polypeptide coded for by the inserted HTLV-III DNA.

21. A method of Claim 20 wherein the cleaving step comprises digesting the HTLV-III cDNA with restriction endonucleases to produce restriction fragments of cDNA.

22. A method of Claim 20 wherein the cleaving step comprises shearing the HTLV-III cDNA to produce cDNA fragments.

23. A method of producing HTLV-III envelope polypeptide, comprising the steps of:

a. cleaving HTLV-III genomic cDNA with the restriction endonuclease SstI;

b. digesting the cleaved cDNA with restriction endonucleases sufficient to generate restriction fragments which encompass at least a portion of the <u>env</u> gene;

c. isolating the restriction fragments;

d. producing DNA fragments of about 200-500 base pairs in length from the restriction fragments;

e. isolating the DNA fragments of about 200-500 base pairs;

f. inserting the isolated fragments into the open reading frame expression vector pMR100 for production of hybrid proteins comprising an env gene product and beta-galactosidase;

g. transforming lac z^- E. coli cells with the vector;

h. plating the transformed cells on MacConkey agar plates, maintaining the plates under conditions sufficient for the formation of colonies and selecting cell colonies exhibiting a red color;

i. culturing transformed cells from the selected colonies under conditions which allow expression of the hybrid protein;

j. obtaining cellular protein from the cultured transformed cells;

k. separating the cellular protein obtained;

l. contacting the separated protein with sera from AIDS patients to identify protein which is immunoreactive with the sera; and

m. isolating the immunoreactive protein.

24. A method of Claim 23, further comprising the step of separating the env gene expression product from the remainder of the hybrid protein.

25. A fusion protein produced by the method of Claim 23.

26. A HTLV-III envelope polypeptide produced by the method of Claim 24.

27. Antibody specifically reactive with HTLV-III envelope polypeptide.

28. An antibody of Claim 27 which is monoclonal.

29. Anibody specifically reactive with HTLV-III polypeptide produced by recombinant DNA techniques.

30. An antibody of Claim 29 which is monoclonal.

31. An immunoassay for the detection of HTLV-III employing antibody which reacts specifically with HTLV-III polypeptide produced by recombinant DNA techniques.

32. An immunoassay for the detection of HTLV-III employing antibody which reacts specifically with HTLV-III envelope polypeptide.

33. An immunoassay of Claim 32 wherein said antibody is monoclonal.

34. A method for detecting the presence of HTLV-III in a bodily fluid comprising the steps of:

a. contacting an immunoadsorbent comprising a solid phase having an antibody which specifically binds HTLV-III polypeptide with the bodily fluid;

b. separating the immunoadsorbent and the fluid;

c. contacting the immunoadsorbent with a labeled antibody which specifically binds HTLV-III polypeptide; and

d. measuring the amount of label associated with the immunoadsorbent to determine the presence of HTLV-III.

35. An assay kit comprising an antibody which reacts specifically iwth HTLV-III polypeptide bound to a solid phase and a labeled antibody which reacts specifically HTLV-III polypeptide.

36. A method of determining the presence of antibodies against HTLV-III in a bodily fluid comprising the steps of:

a. contacting an immunoadsorbent comprising an HTLV-III polypeptide bound to a solid phase with a bodily fluid;

b. separating the immunoadsorbent from the bodily fluid;

c. contacting the immunoadsorbent with a labeled HTLV-III polypeptide; and

d. determining the amount of labeled polypeptide bound to immunoadsorbent as an indication of antibody to HTLV-III.

37. A kit for determining the presence of antibody against HTLV-III in a bodily fluid comprising:

a. an immunoadsorbent comprising a HTLV-III polypeptide bound to a solid phase; and

b. labeled HLTV-III polypeptide.

38. A method of detecting HTLV-III nucleic acid in a bodily fluid comprising the steps of:

a. adsorbing the nucleic acid in a bodily fluid onto an adsorbent;

b. denaturing the adsorbed nucleic acid;

c. contacting the adsorbed nucleic acid with a HTLV-III DNA or RNA probe; and

d. determining if the probe hybridizes with the adsorbed nucleic acid.

39. A method of Claim 38 wherein the bodily fluid is a cell lysate.

40. A hybridoma cell line which produces antibody specifically reactive with HTLV-III envelope polypeptide.

CLONING AND EXPRESSION OF HTLV-III DNA

Abstract

The production of immunoreactive polypeptides from HTLV-III by recombinant DNA methods is disclosed. Such polypeptides can be employed in immunoassays to detect HTLV-III.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Declaration for Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

CLONING AND EXPRESSION OF HTLV-III DNA

the specification of which (check one)

/ / is attached hereto.

/XX/ was filed on October 10, 1984 as Application Serial No. 659,339 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

			Priority Claimed	
(Number)	(Country)	(Day/Month/Year filed)	/ / Yes	/ / No
(Number)	(Country)	(Day/Month/Year filed)	/ / Yes	/ / No
(Number)	(Country)	(Day/Month/Year filed)	/ / Yes	/ / No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.) (Filing date) (Status, patented, pending, abandoned)

(Application Serial No.) (Filing date) (Status, patented, pending, abandoned)

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

I also hereby grant additional Powers of Attorney to the following attorney(s) and/or agent(s) to file and prosecute an international application under the Patent Cooperation Treaty based upon the above-identified application, including a power to meet all designated office requirements for designated states.

David E. Brook	Registration No. 22,592
James M. Smith	Registration No. 28,043
Leo R. Reynolds	Registration No. 20,884

all of Hamilton, Brook, Smith and Reynolds, Two Militia Drive, Lexington, Massachusetts 02173;

and

Send correspondence to: David E. Brook
Hamilton, Brook, Smith & Reynolds
2 Militia Drive, Lexington, MA 02173

Direct telephone calls to: David E. Brook

617-861-6240

Nancy T. Chang — Attorney's Docket No.: CTR84-7

Patent No. or Serial No.: 659,339

Filed or Issued: October 10, 1984

For: CLONING AND EXPRESSION OF HTLV-III DNA

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) and 1.27(c) - SMALL BUSINESS CONCERN

I hereby declare that I am

[ ] the owner of the small business concern identified below:

[X] an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN Centocor, Inc.

ADDRESS OF CONCERN 244 Great Valley Parkway
Malvern, PA 19355

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled CLONING AND EXPRESSION OF HTLV-III DNA by inventor(s) Nancy T. Chang

described in

[ ] the specification filed herewith

[X] application serial no. 659,339, filed October 10, 1984

[ ] patent no. _____, issued _____

[redacted] concern are not exclusive, each individual, [redacted]ion having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9 (d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9 (d) or a nonprofit organization under 37 CFR 1.9(e). *NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME _____

ADDRESS _____

[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATI

NAME _____

ADDRESS _____

[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATI

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Vincent R. Zurawski, Jr.

TITLE OF PERSON OTHER THAN OWNER Executive Vice President and Technical Dire

ADDRESS OF PERSON SIGNING 244 Great Valley Parkway

Malvern PA 19355

SIGNATURE _____ DATE November 6, 1984

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor Nancy T. Chang

Inventor's signature Nancy T. Chang

Date NOV 6. 1984

Residence Paoli, PA 19301

Citizenship Republic of China

Post Office Address 1504 Sugartown Road
Paoli, PA 19301

Full name of second joint inventor, if any

Second Inventor's signature

Date

Residence

Citizenship

Post Office Address

Full name of third joint inventor, if any

Third Inventor's signature

Date

Residence

Citizenship

Post Office Address

Full name of fourth joint inventor, if any

Fourth Inventor's signature

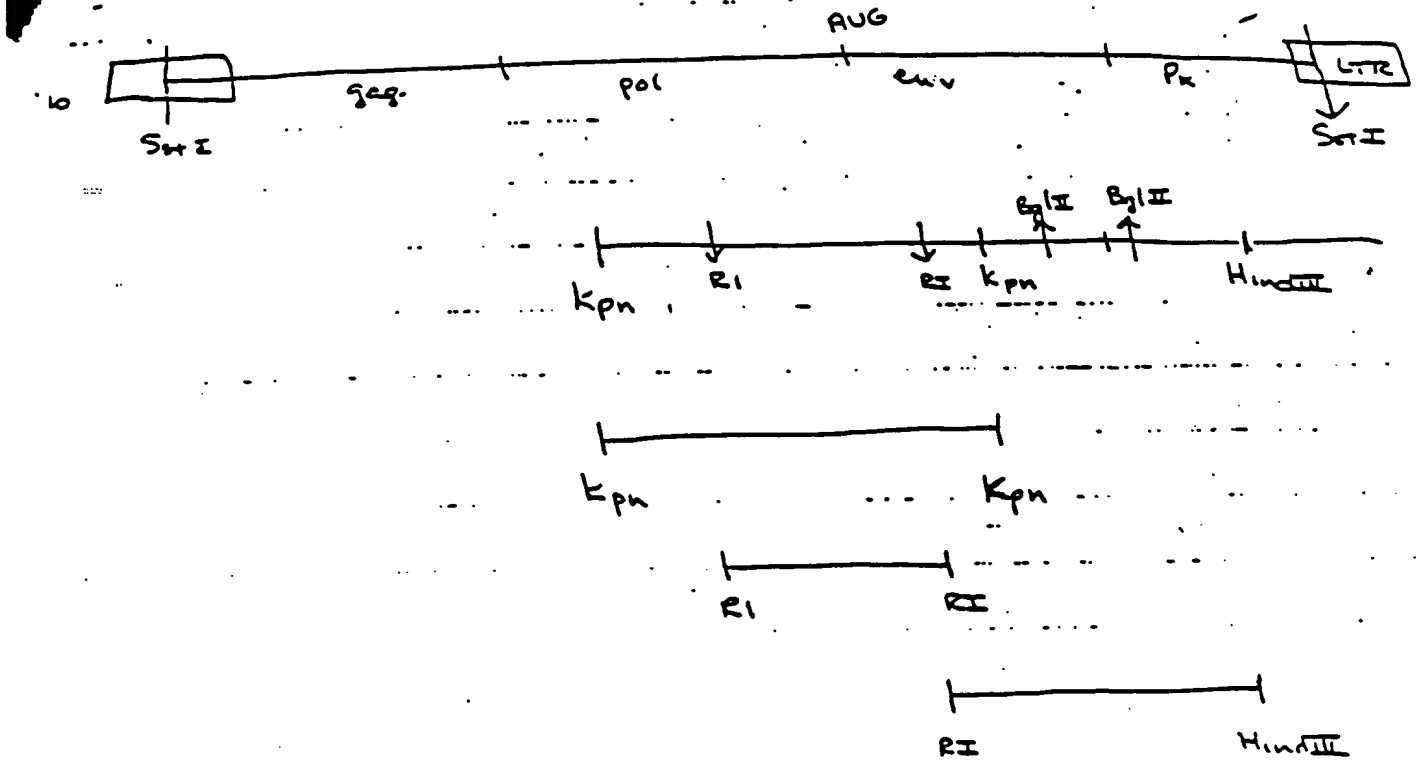
Date

Residence

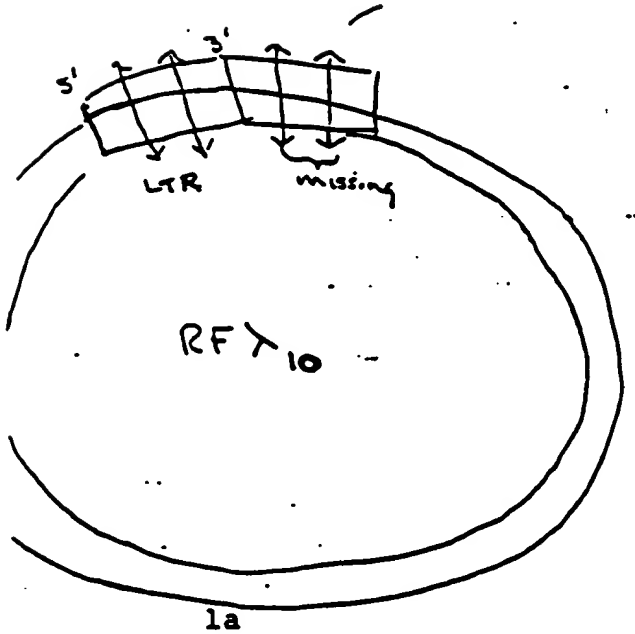
Citizenship

Post Office Address

E59339


AUG
gag
pol
env
LTR
SmaI
SmaI
BglII
BglII
Kpn
R1
RI
Kpn
HindIII
Kpn
Kpn
R1
RI
RI
HindIII

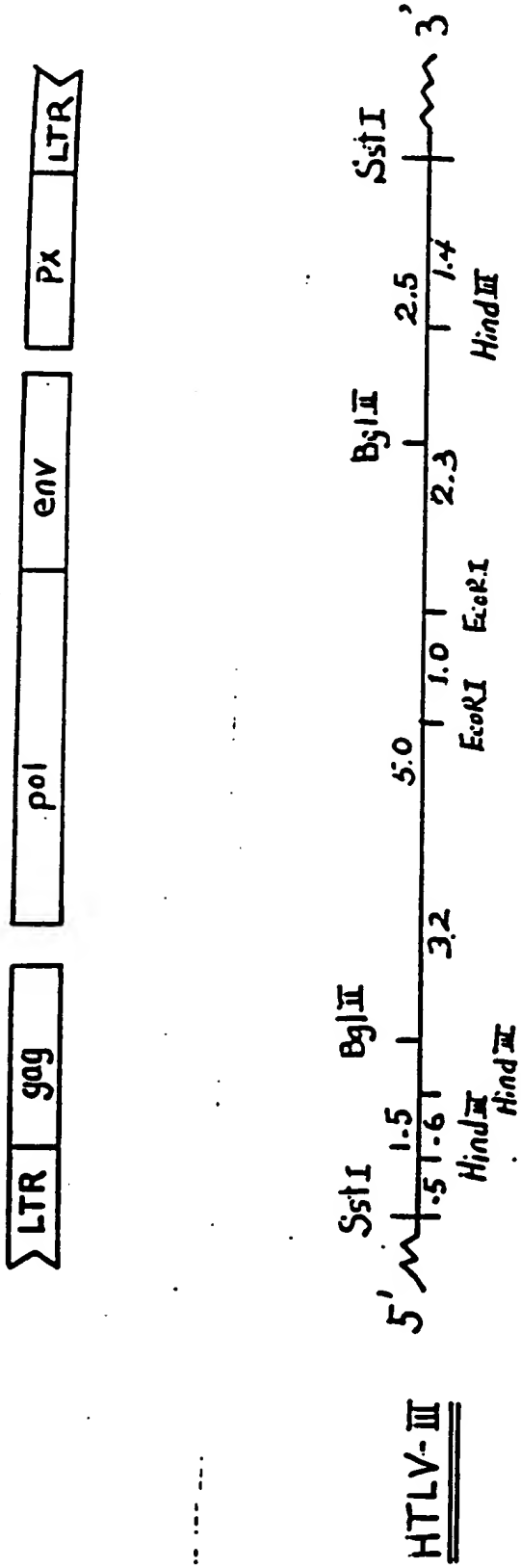
1b


5'
3'
LTR
missing
RF λ10

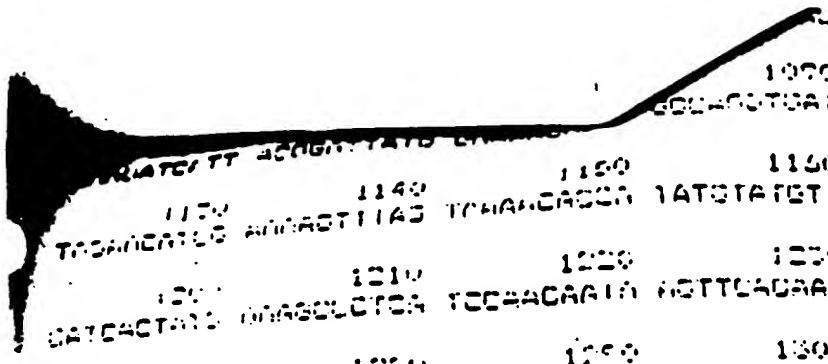
1a

033039

FIGURE 2



```
        80         90                                                                         140
[illegible] ATTACGCAT CATTCAAGTA C[illegible]                    [illegible]TTTAGTC AATCAATA[illegible]

   150        160        170        180        190        200        210
[illegible] AATA[illegible]AAAG G[illegible]AACCTCT A[illegible]C[illegible]A[illegible] GGTACC[illegible]CCA C[illegible]CAAAG[illegible] [illegible]

   220        230        240        250        260        270        280
[illegible] [illegible] [illegible]AGTGCTCG [illegible]TCAGGAAA A[illegible]TTT [illegible]AGATCCAAT AGAT[illegible]

   290        300        310        320        330        340        350
[illegible] A[illegible]AAATA TC[illegible]AGTAA[illegible] [illegible]GCAGAGCAT TCCCTAGTGA [illegible] [illegible]

   360        370        380        390        400        410        420
[illegible] [illegible]AGTA[illegible] AGCTGTGATA AATGTCA[illegible]CT AAAA[illegible]GAGAA GCCATGCATG GACAAC[illegible]ACA

   430        440        450        460        470        480        490
[illegible] [illegible]A[illegible] AACTAGATTG TAGACATTTA GAAG[illegible]AAAAG TTATCCTGGT AGCAG[illegible]

   500        510        520        530        540        550        560
[illegible] [illegible]ATATATAGA A[illegible]AGAACTT ATTCCACCA[illegible] AAACAGGGCA GGAAACACCA TATTTTTTTT

   570        580        590        600        610        620        630
[illegible] AGGAAGA[illegible] CCAGTAAAAA CAATACATAC [illegible]GACAATGCC ACCAATTTCA CCAGTCCTAC

   640        650        660        670        680        690        700
[illegible] GCCTGTTGGT GCCCCGGAAT CAACCAGGAA TTTCCAATTC CCTACAATCC CCAAAGTCAA

   710        720        730        740        750        760        770
TAGTAG AATCTATGAA TAAAGAATTA AAGAAAATTA TAGGACAGGT AAGAGATCAG GCTGAACATC

   780        790        800        810        820        830        840
CACAGC AGTAGAAATG GCAGTATTCA TCCACAATTT TAAAAGAAAA GGGGGGATTG GGGGGTACAG

   850        860        870        880        890        900        910
[illegible]GGGGAA AGAATAGTAG ACATAATAGC AACAGACATA CAAACTAAAG AATTACAAAA ACAAATTACA

   920        930        940        950        960        970        980
[illegible]ATTCAAA ATTTTCGGGT TTATTACAGG GACAGCAGAA ATCCACTTTG GAAAGGACCA GCAAAGCTCC
```



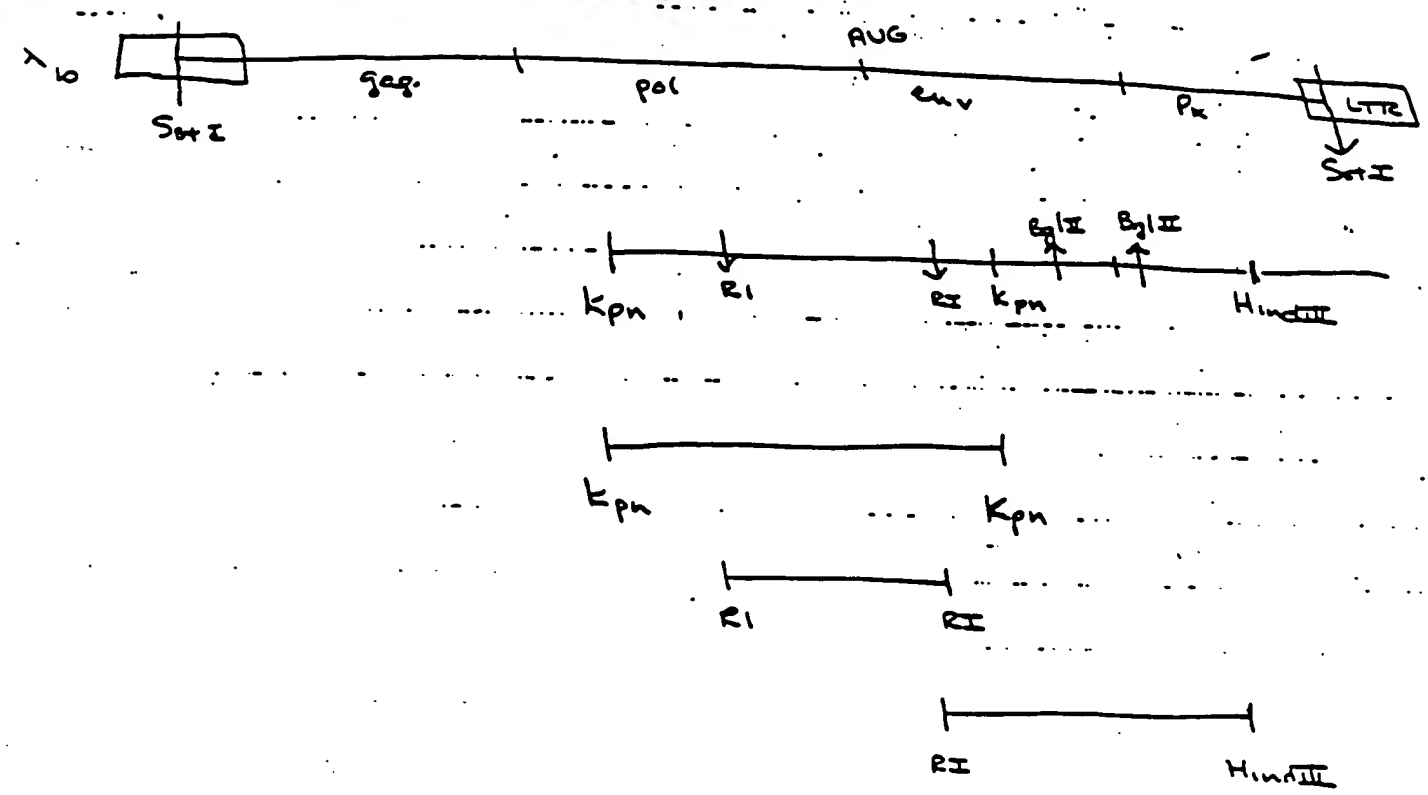
659339

(C)

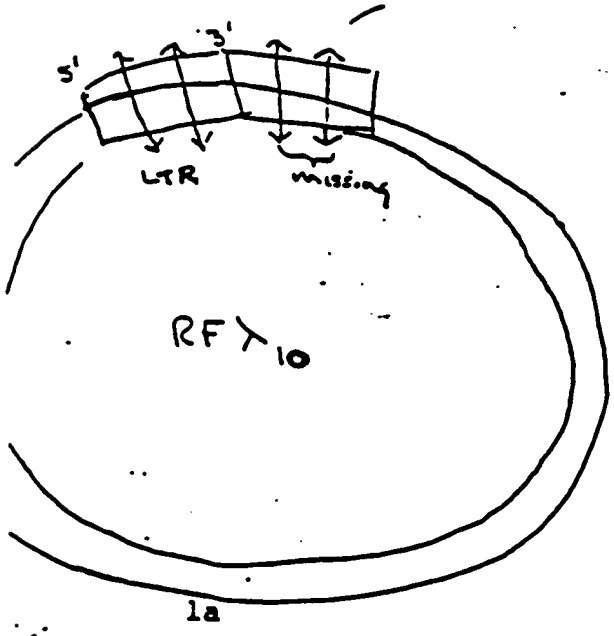
```
      2390       2400       2410       2420       2430       2440       2450
GGAAGCAAG TAACCACCAC TCTATTTTGT GCATCAGATG CTAAAGCATA TGATACAACA GGTACATAAT

      2460       2470       2480       2490       2500       2510       2520
CTGGC A TACATGCCTG TGTACCCACA GACCCCAACC GACAAGAAGT ACTATTCCTA ACTGTGACGG

      2530       2540       2550       2560       2570       2580       2590
AGAATTTTAA GATGTGGAAA AATGACATGG TAGAACAAAT GGATCAGGAT CAAATCATTT TATGGGA A

      2600       2610       2620       2630       2640       2650       2660
AGCCCAAAG CCATGTGTAA AATTAACCCC ACTCTGTGTT ACTTTAAAGT GCACTGC TT CAAAAC GAT

      2670       2680       2690       2700       2710       2720       2730
ACTAATACCA ATAGTAGTAG CGGGAGGATG ATAATGGAGA AAGGAGAGAT GAAAAACTGC TCTTTCAATA

      2740       2750       2760       2770       2780       2790       2800
TCAGGACAAC CATAAGAGGT AAGGTGCAGA AAGAATATGC ATTTTTTTAT AAACTTGATA TAATACCAAT

      2810       2820       2830       2840       2850       2860       2870
AGATAATAAT AGTACCAGCT ATACGTTGAC AAGTTGTAAC ACCTCAGTCA TTACACAGGC CTGTCCAAAG

      2880       2890       2900       2910       2920       2930       2940
GTATCCTTTG AGCCAATTCC CATACATTAT TGTGCCCCGG CTGGTTTTGC GATTCTAAAA TGTAATAATA

      2950       2960       2970       2980       2990       3000       3010
AGACGTTCAA TGGAACAGGA CCATGTACAA ATGTCAGCAC AGTACAATGT ACACATGGAA TTAGGCCAG

      3020       3030       3040       3050       3060       3070       3080
AGTATCAACT CAACTGCTGT TAAATGGCAG TCTAGCAGAA GAAGAGGTAG TAATTAGATC TGTCAATTT

      3090       3100       3110
ACGGACAATG CTAAAACCAT AATAGTACAG CT
```

009559


pMR100
pMR200
1
2
4
103
105
107
117
118
φ
2hr double screen -80°C
10/07/84

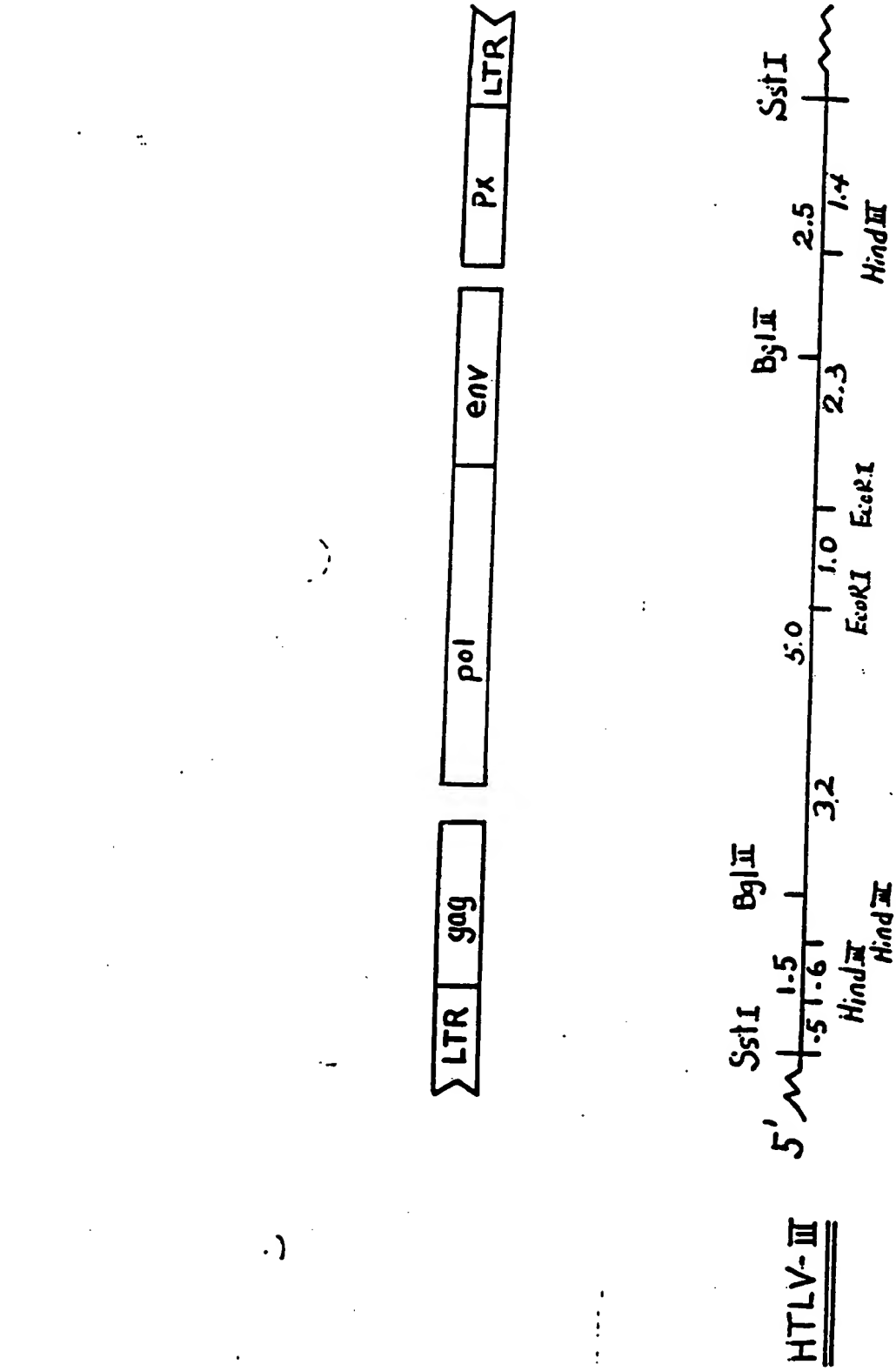

λ10
gag.
pol
AUG
env
Pr
LTR
Sst I
Sst I
Bgl II
Bgl II
Kpn
R1
RI
Kpn
Hind III
Kpn
Kpn
R1
RI
RI
Hind III

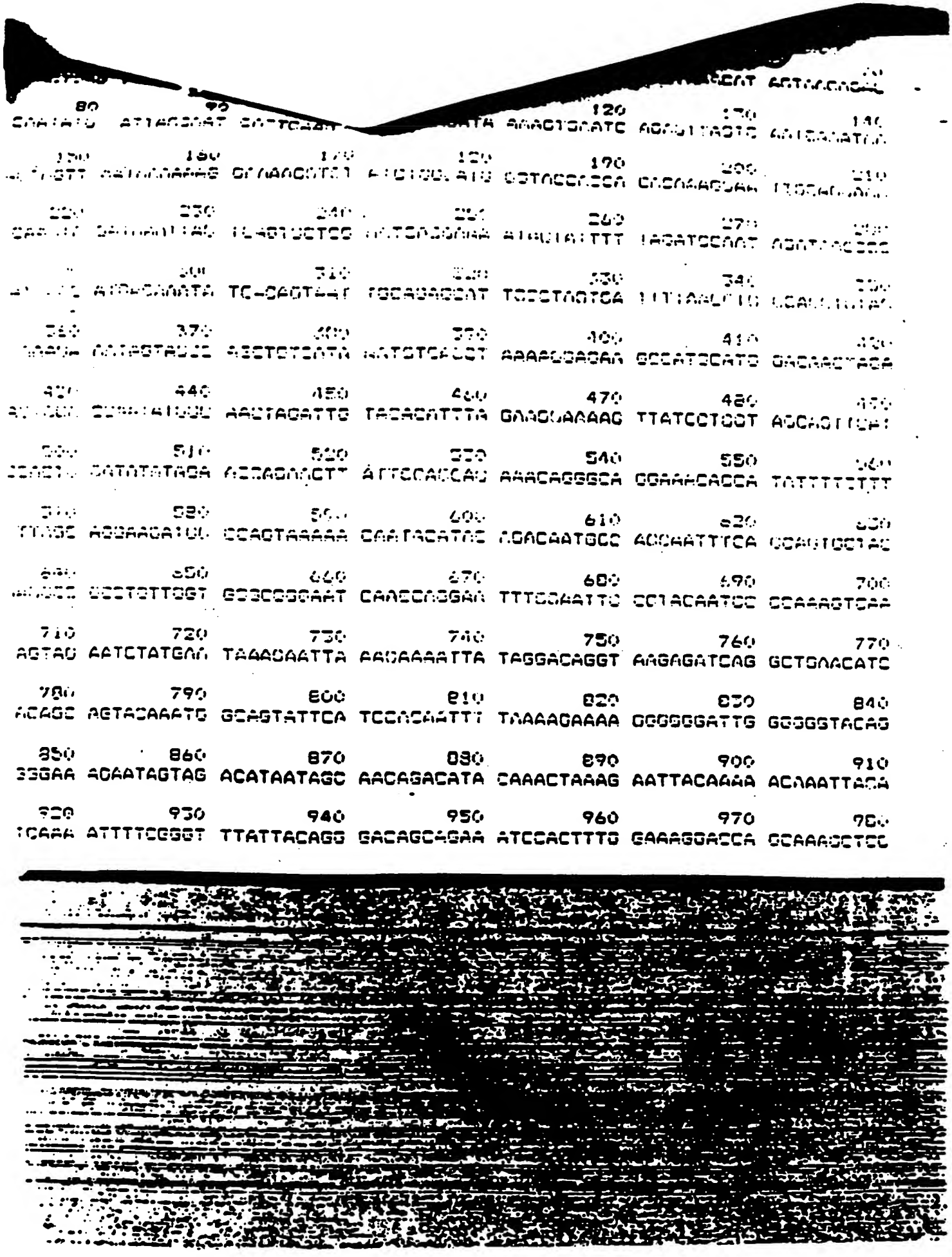
1b

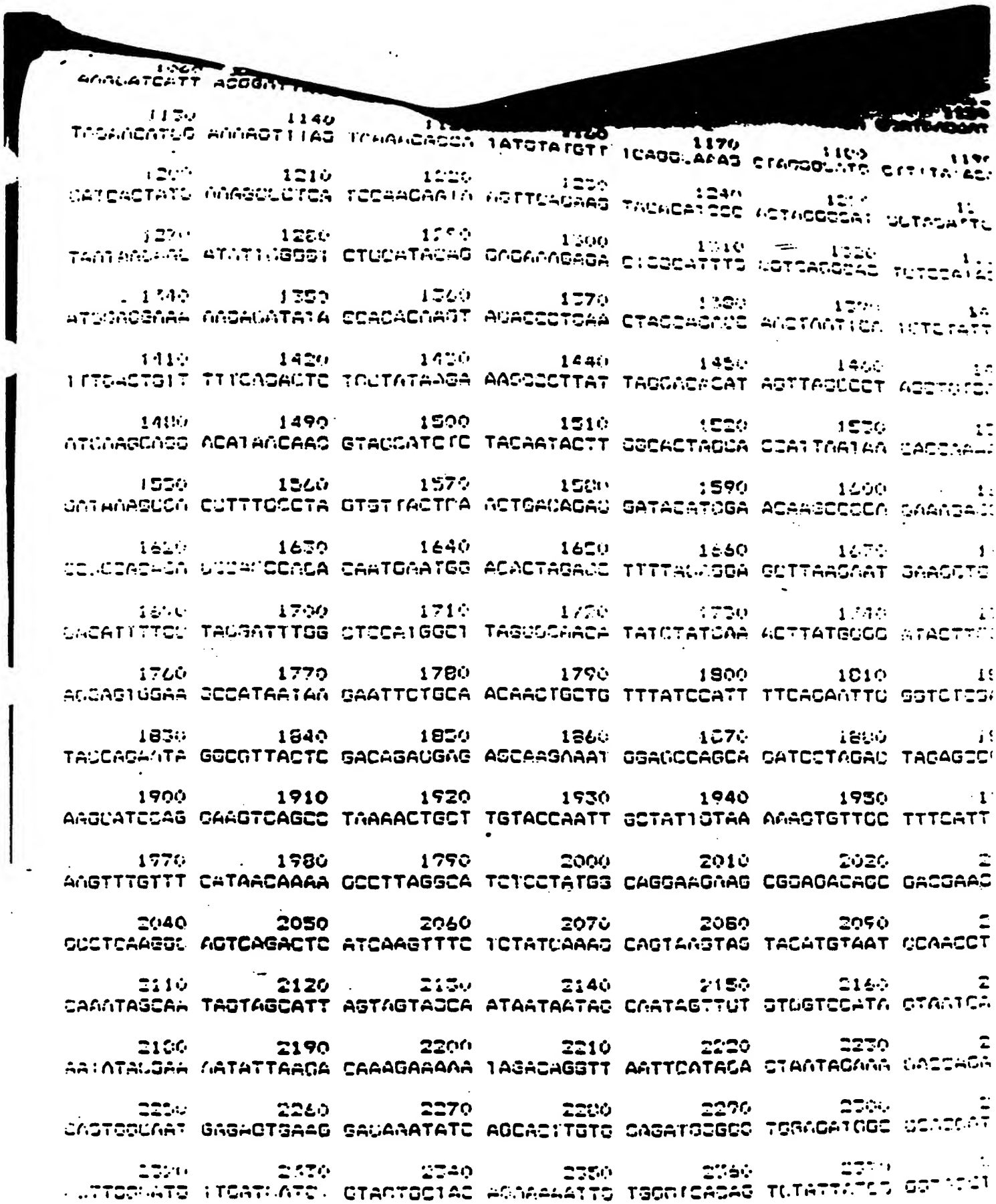

5'
3'
LTR
missing
RF λ10

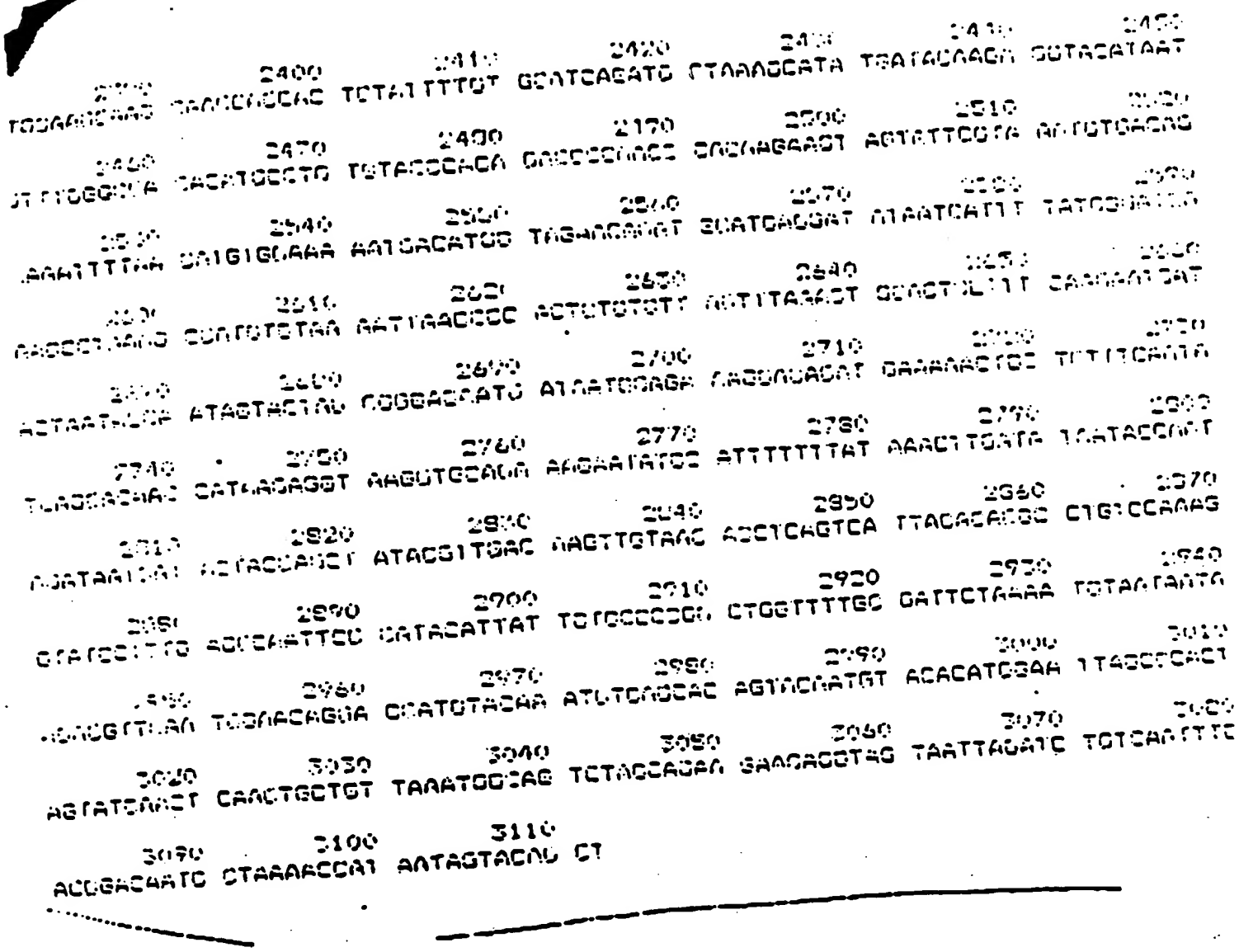
1a

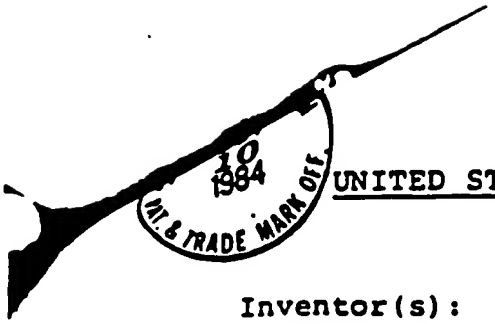
FIGURE 2


LTR
gag
pol
env
PX
LTR
HTLV-III
5'
SstI
1.5
1.5
1.6
HindIII
BglII
3.2
5.0
EcoRI
1.0
EcoRI
BglII
2.3
2.5
HindIII
1.4
SstI
3'






2400
TCTATTTTGT GCATCAGATG CTAAAGCATA TGATAGAAGA GGTACATAAT
2470
CACATGCCTG TGTACCCACA GAGCCCAAGC CAGTAGAACT AGTATTCGTA AATGTGACAG
2540
GAIGIGCAAA AATGACATGG TAGAAGCAGT GCATCAGCAT ATAATCATTT TATCGAATGA
2610
AATTAGCCCC ACTGTGTGTT AGTTTAAACT GCACTGCTTT CAAAAATGAT
2680
ATAATGCAGA AAGAAGACAT GAAAAACTGC TCTTTCAATA
2750
CATAAGAGGT AAGGTCCAGA AAGAATATCC ATTTTTTTTAT AAACTTGATA TAATACCAAT
2820
ATACGTTGAC AAGTTGTAAC ACCTCAGTCA TTACACAAGC CTGTCCAAAG
2890
GATACATTAT TCTGCCCCGC CTGGTTTTGC GATTCTAAAA TGTAATAATG
2960
CTATGTACAA ATGTCAGCAC AGTACAATGT ACACATGGAA TTAGCTCACT
3030
CAACTGCTGT TAAATGCCAG TCTACCAGAA GAAGACGTAG TAATTAGATC TGTCAATTTC
3100
3110
CTAAAACCAT AATAGTACAG CT



UNITED STATES APPLICATION FOR LETTERS PATENT

Inventor(s): Nancy T. Chang

Attorney's Docket No.: CTR84-7

Title: CLONING AND EXPRESSION OF HTLV-III DNA

EXPRESS MAIL Mailing Label No. B15656704

Date of Deposit October 10, 1984

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D. C. 20231.

Gail Munroe
(Typed or printed name of person mailing paper or fee)

Gail Munroe
(Signature of person mailing paper or fee)

American Type Culture Collection
12301 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301)881-2600 Telex: 898-055 ATCCNORTH

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

National Institutes of Health, National Cancer Institute
Building 37, Room 6A17
9000 Rockville Pike
Rockville, Maryland 20205
Attention: Dr. Flossie Wong-Staal

Deposited on Behalf of: National Institute of Health, National Cancer Institute

Identification Reference by Depositor:	ATCC Designation
λ EH-10 recombinant phage clone of HTLV-III in λg & Wes λB	40125
λ EH-5 recombinant phage clone of HTLV-III in λg & Wes λB	40126
λ EH-8 recombinant phage clone of HTLV-III in λg & Wes λB	40127

The deposits were accompanied by: ___ a scientific description ___ a proposed taxonomic description indicated above.

The deposits were received July 30, 1986 by this International Depository Authority and have been accepted.

AT YOUR REQUEST:

___ We will inform you of requests for the strains for 30 years.
_X_ We will not inform you of requests for the strains.
___ The strains are available to the scientific public upon request as of

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years after the date of deposit, and for a period of at least five years after the most recent request for a sample. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above were tested March 4, 1987. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC: Bobbie A. Brandon
(Mrs.) Bobbie A. Brandon, Head, ATCC Patent Depository

Date: March 6, 1987
cc: James A. Oliff, Esq.

Form BP 4/9

P.3

NOV 22 '95 10:33AM ATCC

Documentary Exhibit 12
CHANG ET AL.
Interference No. 103,659

CTN84-7
GAD:esk
2-27-86

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Nancy T. Chang

Serial No.: 659,339

Filed: October 10, 1984

Title: CLONING AND EXPRESSION OF HTLV-III DNA

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to Honorable Commissioner of Patents and Trademarks, Washington, D.C. 20231, on 5-14-86

Hamilton, Brook, Smith & Reynolds

Ellen Kitrosser
Signature

5-14-86
Date

DECLARATION OF NANCY T. CHANG

The Honorable Commissioner
of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Nancy T. Chang, of 7405 Brompton St., Houston, Texas 77025, declare:

1. I am an inventor of the subject matter described and claimed in the above-identified application. When the invention was made, I was

Affidavit Exhibit 2
CHANG ET AL.
Interference No. 103,659

Associate Research Director in Molecular Biology at Centocor, Incorporated, Malvern, Pennsylvania (Centocor), assignee of the subject application. Currently, I am an Associate Professor of Medicine at Baylor College, Houston, Texas.

2. At the time the application was filed, Dr. Robert C. Gallo and Dr. Flossie Wong-Staal were not designated co-inventors when, in fact, they were co-inventors and should be so designated.

3. The above-identified application discloses and claims methods for cloning and expressing subgenomic fragments of HTLV-III cDNA; HTLV-III cDNA fragments and immunoreactive HTLV-III polypeptides encoded thereby; and methods of detecting antibody against HTLV-III employing the polypeptides.

4. The experimental work described in the application began at Centocor upon receipt of genomic HTLV-III DNA from the laboratories of Dr. Gallo and Dr. Wong-Staal. Dr. Gallo and Dr. Wong-Staal supplied a recombinant phage (designated λBH 10) consisting of the genomic HTLV-III cDNA recombined with a phage vector. The HTLV-III cDNA insert was excised from λBH 10 and fragmented and the subgenomic fragments were cloned and expressed in host cell systems as described in the application. All of the experimental work described in the application was done at Centocor, either by me or by laboratory assistants working under my direction and supervision. However, at various times before the experimental work and during its progress, Dr. Gallo, Dr. Wong-Staal and I discussed the strategy

for the cloning and expressing of the viral cDNA. The experimental work proceeded along the lines we discussed; thus Dr. Gallo and Dr. Wong-Staal contributed signficantly to the cloning and expression of the HTLV-III cDNA.

5. On August 22, I prepared a document which described the experimental work accomplished up to that time. The document was sent to Centocor's patent law firm, Hamilton, Brook, Smith & Reynolds (HBS&R), as an "invention disclosure" (Exhibit A). Because all of the work described in the "invention disclosure" document was done at Centocor and because of my incomplete understanding of the law of inventorship, I did not designate Dr. Gallo or Dr. Wong-Staal as "inventor" on this document.

6. Subsequently, Centocor decided to have a patent application prepared and filed by HBS&R. Because of the imminent publication of an article disclosing work relating to the invention, there was great urgency to file the application. On October 8, 1984, I met with Centocor's patent attorneys to supplement information contained in the "invention disclosure" document (Exhibit A) for completing of a patent application. At this meeting, all of my time was devoted to explanation and discussion of the highly technical and complex subject matter necessary to prepare the application. The subject Application, Ser. No. 659,339 was filed on October 10, 1984.

7. On January 23, 1985, a continuation-in-part application was filed to cover additional experi-

mental work which had been done since the earlier application was filed. The inventorship error was repeated.

8. The possibility of an error in inventorship was first raised by Dr. Gallo in a letter to me dated July 25, 1985 (Exhibit B). Shortly thereafter, Centocor management initiated an investigation into the facts surrounding the invention and authorized HBS&R to do the same (Exhibit C). After a preliminary investigation, Centocor management made a tentative response to Dr. Gallo on September 16, 1985 (Exhibit D). However, pursuant to Centocor's stated desire to have the patent legally drawn a thorough investigation was made. During this investigation, I informed HBS&R of the full extent of Dr. Gallo's and Dr. Wong-Staal's collaboration with me regarding conceptual aspects of the claimed subject matter. After consideration, HBS&R concluded that Dr. Gallo and Dr. Wong-Staal should be designated as co-inventors because of their conceptual contributions.

9. My earlier failure to indicate the contributions of Robert C. Gallo and of Flossie Wong-Staal was unintentional.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001

of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Nancy T. Chang

Nancy T. Chang

Feb. 23, 1986

Date

EXHIBIT A

THE CHARACTERIZATION AND PRODUCTION OF HTLV-III GENES AND PROTEINS BY GENETIC ENGINEERING METHODS

Nancy T. Chang

Centocor, Inc.

244 Great Valley Parkway

Malvern, PA 19355

August 22, 1984

Nancy T. Chang
Aug. 27, 1984

8/27/

Witnessed by

Aug 27, 1984

Documentary Exhibit 5
CHANG ET AL.
Interference No. 103,659

Diagnostic and Vaccine Developments for AIDS

Human T Cell Leukemia Virus type III (HTLV-III), also named Lymphoadenopathy Virus (LAV), was isolated from the peripheral blood or lymphoid tissues of patients with Acquired Immune Deficiency Syndrome (AIDS). Recent studies of R. Gallo's group and of L. Montagnier's group indicated that the sera from over 80% of AIDS and pre-AIDS patients contain antibodies specific for the viral envelope and core proteins of HTLV-III. This and other evidence strongly suggests that HTLV-III is the cause of infectious AIDS, giving hopes that the diagnosis, preventive vaccine, and even therapy for AIDS can soon be developed.

Because AIDS can be transmitted through blood transfusions, an assay that detects HTLV-III infection is important not only for diagnosing patients but also for screening blood that might be contaminated with the virus. NIH and several commercial firms, including Centocor, are now developing immunoassay kits employing inactivated, disrupted HTLV-III as the solid-phase immunoadsorbent for the detection of antibodies against HTLV-III antigens in serum or blood.

Genetic Engineering Approach

Another approach for the detection of and the vaccination against HTLV-III infection is the employment of genetic engineering methods. In this approach, the proviral genes integrated into host cell DNA are molecularly cloned. The nucleotide sequence of the molecular cloned provirus is determined. The viral nucleotide sequence information will be directed to design and engineer HTLV-III-specific peptides and DNA probes using recombinant DNA technology or synthetic peptide chemical synthesis methods. These products are then explored for use in the diagnosis of HTLV-III infections by measuring specific antibody to the viral peptides or HTLV-III-specific RNA or DNA. The peptides, especially the gag and env related peptides may also be used as vaccines for the prevention of AIDS.

More specifically, the env and gag genes, which encode the envelope and core proteins of HTLV-III, respectively, are subcloned into various bacterial or mammalian expression vectors. These expression vectors contain all the necessary controlling elements for the production of the fused HTLV-III env gene in recombinant plasmids bearing host cells. Expression of the HTLV-III related peptide in the foreign host cells can be detected by binding of HTLV-III specific antibody in the AIDS patient serum or hyperimmune serum raised against purified virus. Although the env and gag products are of primary interest for diagnostic and vaccine purposes, the other two genes encoded by HTLV-III, pol and Px are important for understanding the biology of this retrovirus. These genes will be studied as well.

The genetic engineering approach offers a few advantages over the conventional one, which involves growing HTLV-III in cell cultures. For example, in the manufacturing process, because viral antigens are not infectious, working personnel are not exposed to the hazardous virus and the facility requirement will be less stringent than that for virus production. Also, the envelope and core proteins are the dominant immunoreactive viral antigens, immunoadsorbents with the purified viral proteins may offer more antibody-adsorbing capacity and higher sensitivity than those with whole virus. Immunoassays employing envelope and core proteins separately can detect antibodies against envelope and against core proteins. The antibody profile (concentrations and proportions) may reveal certain natures of the disease yet to be discovered. Furthermore, a protein vaccine using purified viral proteins (env or core gene product) will not have the risk of viral infectivity.

Centocor's First Footstep in HTLV-III Molecular Biology Work

As soon as we obtained the information in early May, 1984, that HTLV-III was isolated from AIDS patients and shown convincingly to be the cause of AIDS and that antibodies against HTLV-III antigens were found in over 85% of AIDS and Pre-AIDS patients. I decided to use the genetic engineering approach to develop diagnostic assays for AIDS. On May 10, 1984, Tse Wen Chang, Michael Wall and myself went to Biotech Corporation, Rockville, Maryland, to meet Dr. Robert Ting (Chairman of Biotech) to discuss the collaboration between Centocor and Biotech about coating polystyrene beads with inactivated disrupted HTLV-III. In that meeting, I expressed my interest to clone and

express HTLV-III genes and to use the expressed proteins for diagnostic and vaccine products. Dr. Ting was impressed with our expertise in Molecular Biology and introduced me to Dr. Flossie Wong-Staal, a key associate of Dr. Robert Gallo, with whom he had been collaborating on certain aspects of HTLV-III work. Our collaboration with the NCI group started on that day. We returned to Centocor with E. coli clones encoding segments of HTLV-II DNA. At that time, HTLV-III DNA had not been cloned.

The collaboration between Centocor and the NCI group went on very nicely. On July 5, we visited Dr. Wong-Stahl reporting our progress on HTLV-II and proposing our strategy on HTLV-III. We obtained λ clones harboring a segment of HTLV-III DNA on July 20, 1984. Our work on HTLV-III started on that day.

Centocor's Progress Update

We now have E. coli plasmid clones containing various portions or entire genome of HTLV-III. We have sequenced a segment (about 3500 base pairs long) of HTLV-III genome encoding most of the env gene. We have also cloned HTLV-III DNA in several expression host-vector systems and obtained several clones that can be induced to synthesize polypeptides encoded by the inserted HTLV-III DNA. Efforts are being made to test the reactivity of these polypeptides with antibodies from AIDS patients. When we identify clones that produce polypeptides demonstrating good reactivity with the antibodies, we will produce the polypeptide in large quantities and use it in immunoassay development. We also plan to clone and express the gag gene in a few weeks.

Plans are also being made to transfect mammalian cells with the E. coli cloned env and gag DNA's.

The Application of HTLV-III Related Peptides or Proteins

The viral envelope and core related peptides produced by the env and gag clones, either separately or combined, can be coated or conjugated noncovalently or covalently onto solid phase, such as PVC plate or polystyrene beads to be used as immunoadsorbent for antibodies against them. These solid phase immunoadsorbents are the key components in the immunochemical assays for HTLV-III-specific antibodies, using tracers such as goat anti-human immunoglobulin or protein A that are conjugated with radioactive isotopes such as ^{125}I, or enzymes such as peroxidase or alkaline phosphatase.

The proteins can also be used to prepare vaccine against HTLV-III, which should be useful for high-risk populations, such as homosexual males and recipients of frequent blood transfusions. The genetic engineered envelope and core proteins can also be used as an immunogen to prepare monoclonal or polyclonal antibodies. These antibodies can be employed in immunochemical assays for the detection of viral antigens in serum, blood, lymphocytes, or other tissues of AIDS or pre-AIDS patients.

The nucleotide sequences of HTLV-III env and gag genes yield information about the amino acid residue sequences of the envelope and core proteins.

Artificially synthesized segments of polypeptides according to the sequences may offer potential in diagnostic assays and in vaccines.

The cloned HTLV-III and its sequence can also be used to prepare DNA probes for the detection of HTLV-III RNA, proviral DNA, or encoded mRNA in the lymphocytes, or other tissues of patients.

EXHIBIT B

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20205
Building : 37
Room : 6A09
(301) 496-6007

July 25, 1985

Dr. Nancy Chang
Assistant Research Director
Molecular Biology
CENTOCOR
244 Great Valley Parkway
Malvern, PA 19355

Dear Nancy:

We are pleased that our collaborative efforts are making progress. Your synthesis of HTLV-III env gene products using our HTLV-III DNA clone is encouraging. We are beginning to use these in our larger NCI vaccine research development effort.

However, it has come to our attention that some time ago your organization filed a patent on the synthesis and uses of the expressed products from our HTLV-III DNA clones which were designated for collaborative research. We found out that our names are not included on the patent, despite the fact that your use of the clone was indispensable to your part of the effort.

We assume that this was an oversight. We would like to ask that your patent be modified and that our names be added to your patent application. We feel that a formal recognition of our contribution is integral and that the inclusion of our names is only fair.

Sincerely yours,

Robert C. Gallo, M.D.

RCG/PF/bj

cc Dr. Chabner
Dr. DeVita
Dr. Fischinger
→Dr. Harmison
Dr. Sliski
Dr. Wall

Documentary Exhibit 6
CHANG ET AL.
Interference No. 103,659

EXHIBIT C

CENTOCOR

244 GREAT VALLEY PARKWAY. MALVERN. PA 19355. (215) 296-4488
TELEX: 834823 CENTOCORMARN
FAX: 215-644-7558

August 5, 1985

David Brook, Esquire
Hamilton, Brook, Smith & Reynolds
Two Militia Drive
Lexington, Massachusetts 02173

Dear David:

I will respond to Dr. Gallo at the National Institutes of Health upon my return, August 20, 1985. In the meantime please discuss this matter with Nancy Chang regarding the facts surrounding this invention.

I believe Dr. Gallo mixes up inventorship with contribution. This issue is politically sensitive and I may wish to compromise. I will also discuss this with Dr. Lawless at Du Pont. Du Pont is licensed by the government.

Sincerely,

Hubert J.P. Schoemaker for

Hubert J.P. Schoemaker, Ph.D.
President

HJPS:so'h
attachment
cc: Dr. Nancy Chang
Dr. Gregory Lawless

Documentary Exhibit 7
CHANG ET AL.
Interference No. 103,659

244 GREAT VALLEY PARKWAY, MALVERN, PA 19355. (215) 296-4488
TELEX: 834823 CENTOCORMARN
FAX: 215-644-7558

September 16, 1985

Dr. Robert Gallo
National Institutes of Health
9000 Rockville Pike
Building 37
Room 6A09
Bethesda MD 20205

Dear Dr. Gallo:

I have in hand your letter of July 25, 1985 addressed to Dr. Nancy Chang regarding inventorship on the Centocor HTLV-III patents. There is no question that your collaboration was essential to the overall program and, as you know, we have on every occasion, made this fact clear.

In the case of the patent covering the assay development, our lawyers advised us that, under strict inventorship interpretation, your contribution should be referenced in the patent but that you should not appear as an inventor. These rules are quite contrary to the rules for authorship on scientific papers.

We wish to have the patent legally drawn. Anything to strengthen the patent is an advantage. If the lawyers feel your name should be added, we would be not only willing but anxious to have this accomplished.

I would be happy to discuss this matter with you or your representative or arrange to have our patent attorneys visit you in Washington. If you wish to talk to our attorney, please feel free to call David Brook of Hamilton, Brook, Smith & Reynolds directly at 617-861-6240. David does our patent work and his principal client is MIT. He is most qualified in the patent area.

Your work for the government and the community is outstanding. We have attempted to support you to our utmost in the past and will use our best efforts to support you in the future.

Yours very truly,

Hubert J.P. Schoemaker, Ph.D.
President

cc: D. Brook, Esq.
N. Chang, Ph.D.
H. Wall, Chairman

Documentary Exhibit 8
CHANG ET AL.
Interference No. 103,659

COPY

Harold J. McElhinny (Bar No. 66781)
Andrew E. Monach (Bar No. 87891)
Rachel Krevans (Bar No. 116421)
Tamu K. Sudduth (Bar No. 121099)
Morrison & Foerster LLP
345 California Street
San Francisco, California 94104-2675
Telephone: (415) 677-7000
Facsimile: (415) 677-7522

Attorneys for Plaintiff
Chiron Corporation

UNITED STATES DISTRICT COURT

NORTHERN DISTRICT OF CALIFORNIA

Chiron Corporation, Plaintiff, v. Abbott Laboratories, Defendant.	No. C93-4380 MHP **NOTICE OF ENTRY OF STIPULATION FOR DISMISSAL AND ORDER**

TO DEFENDANT AND ITS ATTORNEYS OF RECORD:

PLEASE TAKE NOTICE that the attached Stipulation for Dismissal and Order was entered by this Court on August 30, 1996.

Dated: September 3, 1996

Morrison & Foerster LLP

By: Rachel Krevans
Rachel Krevans
Attorneys for Plaintiff
CHIRON CORPORATION

SEP 25 '97 15:36 FR TO 9212751684 P.02/11

ORIGINAL

[Names and addresses of counsel appear on signature page]

RECEIVED
AUG 19 1996
RICHARD W. WIEKING
CLERK, U.S. DISTRICT COURT
NORTHERN DISTRICT OF CALIFORNIA

FILED
96 AUG 21 8 10 AM
RICHARD W. WIEKING
CLERK
U.S. DISTRICT COURT
NO. DIST. OF CA.

UNITED STATES DISTRICT COURT

NORTHERN DISTRICT OF CALIFORNIA

CHIRON CORPORATION,

Plaintiff,

v.

ABBOTT LABORATORIES,

Defendant.

No. C93-4380 MHP

STIPULATION FOR DISMISSAL AND ~~[PROPOSED]~~ ORDER

AUG 30 1996
ENTERED IN CIVIL DOCKET ____ 19____

STIPULATION FOR DISMISSAL

Chiron Corporation ("Chiron") and Abbott Laboratories ("Abbott") hereby stipulate as follows:

1. This action shall be dismissed with prejudice as to claims for damages incurred before the effective date of the Settlement Agreement entered into by the parties on April 1, 1996 (the "Settlement Agreement") and as to attorneys' fees or costs, and without prejudice as described in paragraph 3 below.

2. Abbott shall not bring any action challenging the validity of Chiron's United States Patent No. 5,156,949.

3. Chiron may sue Abbott for any future alleged infringement of Chiron's United States Patent No. 5,156,949 only in the event of a valid termination of the HIV License and Option

Agreement Among Chiron Corporation and Ortho Diagnostic Systems, Inc. and Abbott Laboratories executed on August 16, 1996, and in accordance with the terms and conditions thereof.

4. Notwithstanding paragraph 3 of the Settlement Agreement, the parties will file only this Stipulation of Dismissal and Order with the United States District Court for the Northern District of California and these documents will not be filed under seal.

SEP 25 '97 15:36 FR TO 9121275168849 P.04/11

5. The Settlement Agreement and this Stipulation for Dismissal and Order shall have no collateral estoppel or res judicata effect as to the National Institutes of Health in Interference No. 103,659 pending in the United States Patent Office.

Dated: August 16, 1996

CHIRON CORPORATION

By: _____

Its: _____

Dated: _____, 1996

ABBOTT LABORATORIES

By: _____

Its: _____

Dated: _____, 1996

Harold J. Mcelhinny (Bar No. 66781)
Andrew E. Monach (Bar No. 87891)
Rachel Krevans (Bar No. 116421)
Tamu K. Sudduth (Bar No. 121099)
Morrison & Foerster LLP
345 California Street
San Francisco, California 94104-2675
Telephone: (415) 677-7000
Facsimile: (415) 677-7522

By: _____
Harold J. McElhinny
Attorneys for Plaintiff
Chiron Corporation

5. The Settlement Agreement and this Stipulation for Dismissal and Order shall have no collateral estoppel or res judicata effect as to the National Institutes of Health in Interference No. 103,659 pending in the United States Patent Office.

Dated: _____, 1996

CHIRON CORPORATION

By: _____

Its: _____

Dated: August 16, 1996

ABBOTT LABORATORIES

By: _____

Its: V.P., Litigation + Gov't Affairs

Dated: _____, 1996

Harold J. Mcelhinny (Bar No. 66781)
Andrew E. Monach (Bar No. 87891)
Rachel Krevans (Bar No. 116421)
Tamu K. Sudduth (Bar No. 121099)
Morrison & Foerster LLP
345 California Street
San Francisco, California 94104-2675
Telephone: (415) 677-7000
Facsimile: (415) 677-7522

By: _____
Harold J. McElhinny
Attorneys for Plaintiff
Chiron Corporation

SEP 25 '97 15:36 FR 10 9121213184849 P.08/11

5. The Settlement Agreement and this Stipulation for Dismissal and Order shall have no collateral estoppel or res judicata effect as to the National Institutes of Health in Interference No. 103,659 pending in the United States Patent Office.

Dated: _____, 1996

CHIRON CORPORATION

By: _____

Its: _____

Dated: _____, 1996

ABBOTT LABORATORIES

By: _____

Its: _____

Dated: _____, 1996

Harold J. Mcelhinny (Bar No. 66781)
Andrew E. Monach (Bar No. 87891)
Rachel Krevans (Bar No. 116421)
Tamu K. Sudduth (Bar No. 121099)
Morrison & Foerster LLP
345 California Street
San Francisco, California 94104-2675
Telephone: (415) 677-7000
Facsimile: (415) 677-7522

By: Rachel Krevans for
Harold J. McElhinny
Attorneys for Plaintiff
Chiron Corporation

Dated: Aug 15, 1996

Dan K. Webb
George C. Lombardi
James F. Hurst
Winston & Strawn
35 West Wacker Drive
Chicago, Illinois 60601
Telephone: (312) 558-5600

Mark E. Barmak
Laura Jonaus Schumacher
Abbott Laboratories
One Abbott Park Road
Abbott Park, Illinois 60064
Telephone: (708) 937-5201

Curtis E. A. Karnow (Bar No. 111648)
Stephen C. Lewis (Bar No. 66590)
Landels, Ripley & Diamond
350 Steuart Street
San Francisco, California 94105-1250
Telephone: (415) 788-5000

By: _____
Dan K. Webb
Attorneys for Defendant
Abbott Laboratories

ORDER

IT IS HEREBY ORDERED THAT:

1. This action is dismissed with prejudice as to claims for damages incurred before the effective date of the Settlement Agreement entered into by the parties on April 1, 1996 (the "Settlement Agreement") and as to attorneys' fees or costs, and without prejudice as described in paragraph 3 below.

2. Abbott shall not bring any action challenging the validity of Chiron's United States Patent No. 5,156,949.

3. Chiron may sue Abbott for any future alleged infringement of Chiron's United States Patent No. 5,156,949 only in the event of a valid termination of the HIV License and Option

AUG 15 '96 18:29 PAGE.05

Agreement Among Chiron Corporation and Ortho Diagnostic Systems, Inc. and Abbott Laboratories executed on August 16, 1996, and in accordance with the terms and conditions thereof.

4. The Settlement Agreement and this Stipulation for Dismissal and Order shall have no collateral estoppel or res judicata effect as to the National Institutes of Health in Interference No. 103,659 pending in the United States Patent Office.

Dated: AUG 27 1996, 1996

Marilyn Hall Patel
U.S. District Court Judge

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of : Nancy Chang et al.

Serial No. : 06/659,339

Filed : October 10,1984

For : CLONING AND EXPRESSION OF HTLV-III DNA

Assistant Commissioner for Patents
Washington, D.C. 20231

PETITION UNDER 37 C.F.R. §1.182

Sir:

Applicants are petitioning under 37 C.F.R. §1.182 to amend U.S.S.N. 06/659,339, now abandoned, to include a claim for benefit of co-pending application U.S.S.N. 06/643,306, filed August 22, 1984, and to add a reference to the pre-filing date deposit of a HTLV-III recombinant phage clone referred to in the specification of U.S.S.N. 06/659,339. A proposed amendment is submitted herewith together with a check in the amount of $130.00 to cover the petition fee.

STATEMENT OF FACTS

1. U.S.S.N. 06/659,339 (the "'339 application") was filed on October 10, 1984. Drs. Nancy Chang, Flossie Wong-Staal

and Robert Gallo are the inventors[1]. It was abandoned in favor of U.S.S.N. 06/693,866 ("the '866 application"), a continuation-in-part application filed on January 23, 1985. The '866 application is pending and is currently involved in Interference No. 102,822 (APJ Andrew Metz).

2. The '339 application is the grand parent application for U.S.S.N. 08/080,387 (the '387 application") filed on June 21, 1993. The '387 application is currently involved in Interference No. 103,659 (APJ Michael Sofocleus). Applicants are the Senior Party. Chiron Corporation is the real party in interest for the Junior Party.

3. U.S.S.N. 06/643,306, directed to Molecular Clones of the Genome of HTLV-III, was filed on August 22, 1984. This application describes the cloning of HTLV-III from an immortalized human T-cell line and the preparation of molecular clone λBH-10. Drs. Flossie Wong-Staal, Robert C. Gallo, Beatrice Hahn and Mikulas Popovic are the inventors. The '339 application was co-pending

---

[1] As filed, the '339 application listed Dr. Nancy Chang as the sole inventor. On May 14, 1986, petitions to change the inventorship to add Dr. Robert Gallo and Dr. Flossie Wong-Staal were filed in the '339 application and in U.S.S.N. 06/693,866, the continuation in part application filed on January 23, 1985. Apparently, the '339 application was abandoned before the petition to change inventorship was acted upon. However, in Paper No. 13, issued November 27, 1987, the PTO examiner changed the inventorship of the '866 application to include Dr. Gallo and Wong-Staal. Pursuant to the Weil v. Fritz, 572 F.2d 856 (C.C.P.A. 1978) and In re Schmidt, 293 F.2d 274 (C.C.P.A. 1961) decisions, amendment of the '866 application was legally effective to change the inventorship of the '339 application. Thus, Drs. Chang, Gallo and Wong-Staal are the legal inventors of the '339 application.

with U.S.S.N. 06/643,306 and shares two common inventors, namely, Drs. Gallo and Wong-Staal.

4. Prior to the filing date of the '339 application, recombinant phage clones harboring HTLV-III DNA designated λBH-5, λBH-8 and λBH-10 were deposited by Dr. Flossie Wong-Staal an inventor of the '339 application. On July 30, 1984 these clones were received by the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, 20852, and accepted for deposit under ATCC accession numbers 40126, 40127 and 40125, respectively. The ATCC form recognizing the deposit and its acceptance is attached as Chang Documentary Exhibit 12. The deposit is in full compliance with PTO rules.

5. Clone λBH-10 was specifically identified in the '339 application as set forth in detail in the attached proposed amendment.

6. On September 14, 1995, the United States District Court for the Northern District of California issued a decision in the action captioned Chiron Corporation v. Abbott Laboratories, Civil Action C-93-4380(MHP). The Applicants were not parties to the action. Abbott Laboratories is a licensee of the Applicants under the Chang applications. The decision is reported at 902 F. Supp. 1103 (N.D.Cal. 1995) (the "California Decision") and is attached as Chang Documentary Exhibit 1.

7. In the California Decision, the court, without the benefit of any expert testimony and on a record which the court characterized as "quite weak", found that the '339 application does

not enable one of ordinary skill to obtain or make the starting material, i.e., the HTLV-III clones (the "starting material finding"). Chang Documentary Exhibit 1, 902 F. Supp at 1126.

8. In the California Decision, the Court noted that Chiron had asserted that the '339 application did not indicate that the inventors possessed a means for making a recombinant clone encoding the env region of HTLV-III. The court made no finding on this issue (the "written description issue"). Chang Documentary Exhibit 1, 902 F. Supp at 1128-1129.

9. In the California Decision, the court again, on a very limited record, found that the '339 application fails to set forth the best mode based upon the absence of an enabling disclosure regarding the starting material, i.e., HTLV-III clones (the "best mode finding"). Chang Documentary Exhibit 1, 902 F. Supp. at 1129.

REASONS FOR GRANTING PETITION

The petition to enter these amendments in the '339 application should be granted because the amendments are in accordance with PTO rules and practice and Federal Circuit precedent and may facilitate resolution of issues in the interferences.

The amendment seeking to add the specific reference to the '306 application is appropriate under 35 U.S.C. § 120. The '306 application was filed by two inventors common to this application and was co-pending. The '306 application describes the

cloning of HTLV-III and the preparation of a molecular clone of HTLV-III used in the '339 application. The amendment seeks to add a specific reference to the earlier filed '306 application. Since the '339 application is abandoned, a petition to the Commissioner is appropriate. Under the authority of Sampson v. Commissioner of Patents, 195 U.S.P.Q. 136 (D.C.D.C., 1976), entry of the amendment to the '339 application is appropriate.

The amendment to the application adding the reference to the deposit of the HTLV-III clone at the ATCC is also proper under In Re Lundak, 773 F.2d 1216 (Fed. Cir. 1985). As the Court noted:

> Constructive reduction to practice does not turn on the question of who has possession of a sample, and thus it does not turn on the inclusion or absence, in the specification as filed of the name and address of who will have possession of the sample on grant of the patent.
>
> * * *
>
> We conclude that .... the insertion of depository data after filing is not new matter under 35 U.S.C. § 132.

773 F.2d at 1223. The Court of Appeals further noted:

> [T]he function of section 112 in ensuring *complete public disclosure* is only violated if the *disclosure* is not complete *at the time it is made public* i.e. at the issue date.

773 F.2d at 1223 (citations omitted).

The entry of these amendments is warranted in equity to address the starting material finding, the written description issue and the best mode finding in the California Decision, which

Chiron will undoubtedly raise in the interference. The California findings are erroneous, particularly in light of the deposit and resulting availability of the starting material, which is specifically identified in the '339 application, and the description in the '306 application of the molecular cloning of the HTLV-III starting material. The entry of the amendments are fully warranted under controlling law. Accordingly, entry of the proposed amendment is fully justified.

CONCLUSION

Applicants respectfully request that the petition be granted and that the amendment to the '339 application be entered to protect Applicants' patent rights.

AUTHORIZATION

The Assistant Commissioner is hereby authorized to charge any additional fees which may be required in this application,

including a petition fee, to Deposit Account No. 13-4500, Order No. 1436-4094.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

By: Eugene Moroz
Eugene Moroz
Reg. No. 25,237

Of Counsel:

William S. Feiler
M. Caragh Noone
Dorothy R. Auth
MORGAN & FINNEGAN, L.L.P.
345 Park Avenue
New York, New York 10154
(212)758-4800
(212)751-6849 (FAX)

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as Express Mail in an envelope addressed to: the Assistant Commissioner for Patents, Washington, D.C., 20231, on February 20, 1996.

Dated: February 20, 1996 By: Eugene Moroz

Eugene Moroz

198026_1

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER OF
PATENTS AND TRADEMARKS
Washington, D.C. 20231

RECEIVED
DOCKET DEPT.

Paper No. 10

EUGENE MOROZ
MORGAN & FINNEGAN
345 PARK AVENUE
NEW YORK, NEW YORK 10154

3 1996
Morgan & Finnegan
COPY MAILED
29 1996
DIRECTOR'S PETITIONS
AND PATENTS

In re Application of :
Nancy Chang :
Application No. 06/659,339 : ON PETITION
Filed: October 10, 1984 :
Attorney Docket No. CTR84-7 :

This is a decision on the petition under 37 CFR 1.182, filed February 22, 1996, to amend the above-identified abandoned application by: (1) insertion of a reference under 35 USC 120 to application No. 06/643,306, filed August 22, 1984, and (2) insertion of a reference to the pre-filing date deposit of a recombinant phage clone, λBH-10, harboring HTLV-III DNA.

The petition to insert a reference under 35 USC 120 to application No. 06/643,306, filed August 22, 1984, is **GRANTED**.

The petition to insert a reference to the deposit of a recombinant phage clone, λBH-10, harboring HTLV-III DNA is **DISMISSED**.

Any request for reconsideration of this decision must be submitted within TWO (2) MONTHS from the mail date of this decision. Extensions of time under 37 CFR 1.136(a) are permitted. The reconsideration request should include a cover letter entitled "Renewed Petition under 37 CFR 1.182."

Rej - 2 mos.

CASE 1436-4694 ATTY EM
DUE DATE May 29 1996 ✓
STATUTORY DATE September 29, 1996 ✓
BY KB

BACKGROUND

Although instant application No. 06/659,339 ('339) was pending from October 10, 1984, through March 15, 1987, petitioner did not perfect a claim of domestic priority under 35 USC 120 for benefit of Application No. 06/643,306 ('306) which was pending from August 22, 1984, through July 14, 1987, during the pendency of the '339 application. The '306 application as filed named Robert C. Gallo (Gallo), Flossie Wang-Stall (Stall), Beatrice Hahn, and Mikulas Popovic as inventors. As filed, the '339 application named Nancy T. Chang (Chang) as sole inventor. On May 16, 1986, a petition to correct inventorship under 37 CFR 1.48 was filed, which sought to add Gallo and Stall as joint inventors with Chang. The declaration under 37 CFR 1.63 filed with the petition failed to include the claim for benefit under 35 USC 120 of the earlier '306 application, and the dates of the signatures of the newly added inventors. This petition was apparently never treated by the examiner during prosecution of the '339 application.

Moreover, although a biological material allegedly mentioned in the '339 disclosure had been previously made the subject of a deposit, the '339 specification, as filed, failed to refer to that deposit. During Chiron Corporation v. Abbott Laboratories, 902 F.Supp. 1103 (N.D. Cal. 1995), issues were raised regarding the sufficiency of the disclosure of the '339 application, under 35 USC 112. Petitioner seeks to amend the specification to perfect the claim for domestic priority, as well as to amend the specification to make reference to the deposit.

STATUTE AND REGULATION

35 USC 112, first paragraph, states:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such dull, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set

connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

35 USC 114 states:

> The Commissioner may require the applicant to furnish a model of convenient size to exhibit advantageously the several parts of the invention.
> When the invention relates to a composition of matter, the Commissioner may require the applicant to furnish specimens or ingredients for the purpose of inspection or experiment.

35 USC 115 states, in part:

> The applicant shall make oath that he believes himself to be the original and first inventor of the process, machine, manufacture, or composition of matter for which he solicits a patent; and shall state of what country he is a citizen.

35 USC 116 states, in part:

> When an invention is made by two or more persons jointly, they shall apply for patent jointly and each make the required oath, except as otherwise provided in this title. Inventors may apply for a patent jointly even though (1) they did not physically work together or at the same time, (2) each did not make the same type or amount of contribution, or (3) each did not make a contribution to the subject matter of every claim of the patent. . . .
> Whenever through error a person is named in an application for patent as the inventor, or through an error an inventor is not named in an application, and such error arose without any deceptive intention on his part, the Commissioner may permit the application to be amended accordingly, under such terms as he prescribes.

35 USC 120 states:

> An applicant for patent for an invention disclosed in the manner provided by the first paragraph of section 112 of this title in an application previously filed in the United States, or as provided by section 363 of this title, which is filed by an inventor or inventors named in the previously filed application shall have the same effect, as to such invention, as though filed on the date of the prior application, if filed before the patenting or abandonment or of termination of proceedings on the first application or on an application similarly entitled to the benefit of the filing date of the first application and if it contains or is amended to contain a specific reference to the earlier filed application.

35 USC 131 states:

The Commissioner shall cause an examination to be made of the application and the alleged new invention; and if on such examination it appears that the applicant is entitled to a patent under the law, the Commissioner shall issue a patent therefor.

35 USC 132 states:

> Whenever, on examination, any claim for a patent is rejected, or any objection or requirement is made, the Commissioner shall notify the applicant thereof, stating the reasons for such rejection, or objection or requirement, together with such information and references as may be useful in judging of the propriety of continuing the prosecution of his application and if after receiving such notice, the applicant persists in his claim for a patent, with or without amendment, the application shall be reexamined. **No amendment shall introduce new matter into the disclosure of the invention** [emphasis added].

35 USC 133 states:

> Upon the failure of the applicant to prosecute the application within six months after any action therein, of which notice has been given or mailed to the applicant, or within such shorter time, not less than thirty days, as fixed by the Commissioner in such action, the application shall be regarded as abandoned, unless it be shown to the satisfaction of the Commissioner that such delay was unavoidable.

37 CFR 1.48 provides, in part:

> (a) If the correct inventor or inventors are not named in a nonprovisional application through error without any deceptive intention on the part of the actual inventor or inventors, the application may be amended to name only the actual inventor or inventors. Such amendment must be diligently made and must be accompanied by:
> (1) a petition including a statement of facts verified by the original named inventor or inventors establishing when the error without deceptive intention was discovered and how it occurred;
> (2) an oath or declaration by each actual inventor or inventors as required by § 1.63;
> (3) the fee set forth in § 1.17(h); and
> (4) the written consent of any assignee. When the application is involved in an interference, the petition shall comply with the requirements of this section and shall be accompanied by a motion under § 1.634.

37 CFR 1.63 provides, in part:

> (a) An oath or declaration filed under § 1.51(a)(1)(ii) as a part of a nonprovisional application must:
> (1) Be executed in accordance with either §§ 1.66 or 1.68;
> (2) Identify the specification to which it is directed;

(3) Identify each inventor and the residence and country of citizenship of each inventor; and

(4) State whether the inventor is a sole or joint inventor of the invention claimed.

(b) In addition to meeting the requirements of paragraph (a), the oath or declaration must state that the person making the oath or declaration:

(1) Has reviewed and understands the contents of the specification, including the claims, as amended by any amendment specifically referred to in the oath or declaration ;

(2) Believes the named inventor or inventors to be the original and first inventor or inventors of the subject matter which is claimed and for which a patent is sought; and

(3) Acknowledges the duty to disclose to the Office all information known to the person to be material to patentability as defined in § 1.56. . . .

(d) In any continuation-in-part application filed under the conditions specified in 35 U.S.C. § 120 which discloses and claims subject matter in addition to that disclosed in the prior copending application, the oath or declaration must also state that the person making the oath or declaration acknowledges the duty to disclose to the Office all information known to the person to be material to patentability as defined in § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

37 CFR 1.115 provides, in part:

The applicant may amend before or after the first examination and action and also before the second or subsequent examination or reconsideration as specified in § 1.112 or when and as specifically required by the examiner.

37 CFR 1.118 states in pertinent part:

> (a) No amendment shall introduce new matter into the disclosure of an application after the filing date of the application (§ 1.53(b)). All amendments to the specification, including the claims, and the drawings filed after the filing date of the application must conform to at least one of them as it was at the time of the filing of the application. Matter not found in either, involving a departure from or an addition to the original disclosure cannot be added to the application after its filing date even though supported by an oath or declaration in accordance with § 1.63 or § 1.67 filed after the filing date of the application.
>
> (b) If it is determined that an amendment filed after the filing date of the application introduces new matter, claims containing new matter will be rejected and deletion of the new matter in the specification and drawings will be required even if the amendment is accompanied by an oath or declaration in accordance with § 1.63 or § 1.67.

OPINION

With regard to the request to amend the above-identified abandoned application by insertion of reference under 35 USC 120 to application No. 06/643,306, filed August 22, 1984:

Petitioner relies upon Sampson v. Commissioner of Patents, 195 USPQ 136 (D.D.C. 1976), as support for amending the abandoned '339 application to include a specific reference to the prior co-pending '306 application, in order to perfect a claim for benefit of the '306 filing date under 35 USC 120. Petitioner's noting Weil v. Fritz, 582 F.2d 856, 196 USPQ 600 (CCPA 1978) is apt, inasmuch as that decision considered that a failure to amend the inventive entity during prosecution, as well as a deficiency in the oath or declaration proffered with that amendment, would not be fatal to a claim for benefit of the earlier application's

filing date under 35 USC 120. Id.[1] Thus, with at least one inventor in common to both the '339 and '306 applications, 35 USC 120 is satisfied to the extent requested by petitioner. Petitioner is reminded, however, of the distinction between simply *claiming* benefit under 35 USC 120 as here, and being entitled to or *accorded* benefit under 35 USC 120. See In re Chu, 66 F.3d 292, 36 USPQ2d 1089 (Fed. Cir. 1995). Since, as held in Sampson, 35 USC 120 makes no distinction between active and abandoned applications, and there is no 35 USC 132 bar to this limited amendment, the request to amend the '339 application by inserting a specific reference to the '306 application No. and its filing date is granted.

With regard to the request to amend the above-identified abandoned application by insertion of a reference to the pre-filing date deposit of a recombinant phage clone, λBH-10, harboring HTLV-III DNA:

Petitioner relies upon In re Lundak, 773 F.2d 1216, 227 USPQ 90 (Fed. Cir. 1985), as support for amending the abandoned '339 application to include a reference to the deposit of the HTLV-III clone at the American Type Culture Collection (ATCC). However, neither Sampson nor Lundak stand for the proposition that an abandoned application may be amended to include for the first time a specific reference to a deposit of biological material.

The court in Sampson held that the patent statutes do not bar amendments to an abandoned application to include the technical information required to obtain a benefit under 35 USC 120. Inasmuch as this latter amendment is unrelated to references under 35 USC 120, Sampson does not authorize amendment of an abandoned application to include information concerning biological deposits. Put simply, Sampson does not authorize the Patent and Trademark Office (Office) to permit an applicant to change the invention disclosed to or introduce a concept not previously present in the specification of an abandoned

---

[1] An applicant's oath or declaration is not a requirement under 35 USC 120, but rather a requirement under 35 USC 115. Consequently, its sufficiency is immaterial to satisfaction of 35 USC 120. Id.

application, or to continue prosecution of an abandoned application.

Petitioner's reliance on Lundak is likewise misplaced. The specification at issue in Lundak, *as filed*, contained a specific reference to a deposit of the biological material, and was also held to otherwise satisfy the requirements of 35 USC 112, first paragraph. 775 F.2d at 1223, 227 USPQ at 95. The court held that merely inserting *updated* or *clarified* deposit data into that specification after filing was held not to be new matter (*i.e.*, the inserted data added nothing to the written description), and, as such, the insertions did not enlarge or limit the original disclosure in violation of 35 USC 132. Id., 227 USPQ at 96. The '339 specification, *as filed*, is entirely silent as to any deposit of any biological material at any location; the '339 specification contained no hint of a reference to a deposit. Petitioner has not shown why the proposed insertion of deposit data is not precisely "the shape of new matter against which section 132 was designed to guard". Id., 227 USPQ at 96.

Finally, Lundak involved a pending application, where petitioner seeks to amend the specification of an abandoned application. While a reference to a deposit of biological material in the specification does not create any presumption that such material is necessary to satisfy the provisions of 35 U.S.C. § 112 (37 CFR 1.802(c)), the purpose of the rules of practice and procedures that provide for the deposit of biological material is to facilitate compliance with 35 U.S.C. § 112 in instances in which words alone cannot sufficiently describe the invention in a reproducible manner. See MPEP 2402. An amendment to the specification of a pending application must be considered by the examiner for new matter pursuant to 35 USC 132; however, the examination of an application pursuant to 35 USC 131 *et seq.* involves pending applications. Thus, the requested amendment of this abandoned application by petition would operate to evade the examination of such amendment for new matter pursuant to 35 USC 131 and 132.

The findings of law in Chiron that the '339 specification as filed failed to satisfy the enablement and best mode requirements of 35 USC 112, first paragraph, and the finding of fact that the '339 specification as filed made no reference to a deposit, are

directly opposite to the findings of law and fact in Lundak. Consequently, the proffered insertion is precluded by 35 USC 132.

Petitioner alleges the specific mention of λBH-10 in the '339 application. Assuming, *arguendo*, that petitioner's allegation is correct, petitioner has not shown, nor is it apparent, how this constitutes a reasonably precise reference to a *deposit* of same. Consequently, the latter proposed amendment does not amount to a mere change in wording. See In re Fouche, 439 F.2d 1237, 169 USPQ 429 (CCPA 1971). As such, the latter amendment which seeks to now add to the specification a reference to a deposit would be contrary to Sampson, Lundak, and 35 USC 132. See also In re Hawkins, 486 F.2d 579, 179 USPQ 163 (CCPA 1973).

Unlike the above-permitted amendment, which is merely a permissible 35 USC 120 statement, this latter amendment impermissibly attempts to incorporate by reference subject matter elsewhere set forth. Since the concept of deposit is being newly introduced, subsequent to the filing date, this may constitute the addition of new matter, which is prohibited by statute (35 USC 132) and regulation (37 CFR 1.118). Petitioner's attention is directed to Dart Industries v. Banner, 636 F.2d 684, 207 USPQ 273 (C.A.D.C. 1980), where the court drew a distinction between a permissible 35 USC 120 statement, and the impermissible introduction of new matter by way of incorporation by reference. The court specifically stated:

> Nothing in section 120 itself operates to carry forward any disclosure from an earlier application. In re deSeversky, supra at 674, 177 USPQ at 146-147. Section 120 contains no magical disclosure-augmenting powers able to pierce new matter barriers. It cannot, therefore "limit" the absolute and express prohibition against new matter contained in section 251 [or 132]. Id. at 688, 207 USPQ at 276.

Thus, assuming, *arguendo*, that Sampson authorizes the amendment of an abandoned application beyond the mere inclusion of a 35 USC 120 statement, 35 USC 132 remains an absolute limitation as to the scope and content of such an amendment. Consequently, granting the request to insert a 35 USC 120 statement, would in no way be inconsistent with dismissing the request to insert

deposit information, which here, may constitute the insertion of new matter. Granting the former request does not violate 35 USC 132 by carrying forward any disclosure of the '306 application into the '339 application, or by augmenting the '339 disclosure. Sampson at 137. Therefore, the request to amend the specification by insertion of a reference to a deposit of any biological material, including λBH-10, is dismissed.

Further correspondence with respect to this matter should be addressed as follows:

By mail: Assistant Commissioner for Patents
Box DAC
Washington, D.C. 20231

By FAX: (703) 308-6916
Attn: Office of Petitions

By hand: One Crystal Park, Suite 520
2011 Crystal Drive
Arlington, VA

This application is being forwarded to the Office of the Director, Examining Group 1800, for entry, *in part*, of the amendment received February 22, 1996. Specifically, the 35 USC 120 statement will be inserted into the specification at page 1, first sentence.

Thereafter, this application will be returned to the Office of Petitions.

Telephone inquiries relative to this decision should be directed to Brian Hearn, Office of Petitions, at (703) 305-9282.

Abraham Hershkovitz, Director
Office of Petitions
Office of the Deputy Assistant Commissioner
for Patent Policy and Projects
BH/rwb

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	:	Nancy Chang et al.
Serial No.	:	06/659,339
Filed	:	October 10,1984
For	:	CLONING AND EXPRESSION OF HTLV-III DNA

Assistant Commissioner for Patents
Box DAC
Washington, D.C. 20231

REQUEST FOR RECONSIDERATION OF THE MARCH 29, 1996 DECISION DISMISSING APPLICANTS' PETITION PURSUANT TO 37 C.F.R. §1.182 TO ADD A REFERENCE TO A PRE-FILING DATE DEPOSIT

Sir:

Applicants respectfully request reconsideration of the March 26, 1996 decision dismissing their petition to insert a reference to the pre-filing date deposit of a recombinant phage clone harboring HTLV-III DNA, λBH-10, which is specifically identified in the specification of U.S.S.N. 06/659,339.[1]

Applicants' submit that for the reasons set forth below and those in their petition filed February 20, 1996, amendment of U.S.S.N. 06/659,339, now abandoned, to add the reference to the deposit is appropriate pursuant to the governing case law, the

[1] Applicants' agree with the decision to grant their petition to insert a reference under 35 U.S.C. 120 to application No. 06/643,306, filed August 22, 1984. Thus, reconsideration of this part of the decision is not sought.

Patent and Trademark Office ("PTO") rules regarding deposit of biological materials, 37 C.F.R. §§1.801-1.809, and the Manual of Patent Examining Procedure ("MPEP").

REASONS FOR RECONSIDERING THE DECISION AND GRANTING THE PETITION

Applicants respectfully submit that two positions taken in the March 29, 1996 Decision (the "Decision") are not supported by either the PTO rules and procedure or Federal Circuit precedent. Thus, they should be reconsidered.

1. Addition Of A Reference To A Deposit Is Proper Where The Biological Material Is Specifically Identified In The Specification As Filed

First, Applicants respectfully submit that, contrary to the position in the Decision (pp. 8-10), there is no requirement in either the PTO rules or examining procedure or In re Lundak, 773 F.2d 1216 (Fed. Cir. 1985) that a specification as filed must refer to a deposit of a biological material in order to add a reference to the date, depository name and accession number of the deposited material. Indeed, the deposit rules, 37 CFR §§1.801-1.809, and the MPEP make clear that a post-filing date deposit may be made and/or a reference to deposit data added as long as the biological material was specifically identified in the Application as filed. The PTO rules state:

> Whenever a biological material is specifically identified in an application for patent as filed, an original deposit thereof may be made at any time before filing the application for

> patent or, subject to §1.809, during pendency of the application for patent.

37 CFR §1.804(a) (emphasis added). See also 37 CFR 1.809(d) There is no requirement that the deposit be referenced in the specification. As the MPEP explains:

> 37 CFR 1.804(a) specifies not only a permissible time frame for making an original deposit, but also specifies that the biological material deposited must be specifically identified in the application as filed. The requirement for a specific identification is consistent with the description requirement of the first paragraph of 35 USC 112 and provides an antecedent basis for the biological material which either has been or will be deposited before the patent is granted.

MPEP §2406.01 (emphasis added). Thus, while the biological material must be identified and referenced in the specification, the existence of a deposit need not be mentioned.

Indeed, the MPEP specifically distinguishes between the permissible addition of a reference to a deposit of an identified biological material and the impermissible addition of a reference to the biological material itself, which is prohibited as new matter under 35 USC §132:

> It should be noted, however, that reference to a biological material present in an application upon filing, may form the basis for making a deposit, where required, after the filing date of a given application, but that the reference to the biological material itself, cannot be added after filing without risking the prohibited introduction of new matter.

35 USC §132. MPEP §2404.03

In the instant situation, Applicants seek only to add a reference to the pre-filing date deposit of a biological material, clone λBH-10, which was specifically identified in the specification as filed. Indeed, as discussed in the attached Amendment and Declaration of Dr. Flossie Wong-Staal ("Wong-Staal Declaration", attached as Ex. 1), λBH-10 clones, which were referred to in the '339 specification as "lambda 10 clones" were specifically identified and described in the '339 specification on page 3, lines 28-30, page 8, lines 33 to page 9, line 1, and page 9, lines 3-8 of the '339 application as filed. Additionally, restriction maps of clone λBH-10, showing restriction enzyme sites present in this clone are found at Figs. 1a, 1b and 2 of the '339 application as filed. See Declaration of Dr. Wong-Staal, ¶10.

Moreover, Dr. Wong-Staal's declaration makes clear that the λBH-10 clones deposited more than two months before the filing date of the '339 application are the same as those identified and described in the '339 specification. Finally, the Wong-Staal declaration and Amendment establish that the deposit of clone λBH-10 was made in full compliance with the deposit rules of the PTO, 37 CFR §§1.801-1.809. See Wong-Staal Decl., ¶¶5-8. See also Amendment, pp. 3-5, attached as Ex. 2. Thus, pursuant to 37 CFR §1.802, 1.804 and 1.809(d) and MPEP §§2406.01 and 2404.03, amendment of the '339 specification to include a reference to the deposit of clone λBH-10 is proper.

Applicants note that the Decision appears to interpret the Lundak case to authorize addition of deposit data to a specification only where the deposit itself was referenced in the specification. Applicants' respectfully submit that this is an improperly narrow interpretation of Lundak. As discussed above, such an interpretation would be contrary to the deposit rules and the MPEP. Additionally, the issue before the Federal Circuit in Lundak was not whether Lundak could "update" or "clarify" his deposit data. Rather, as the Federal Circuit noted, the PTO had argued "that a post-filing deposit is barred as new matter, as is the insertion into the specification of reference to such deposit." 773 F.2d at 1222. The Federal Circuit rejected this argument, holding that "the insertion of depository data after filing is not new matter under 35 USC §132." Thus, Applicants respectfully submit that it is improper to interpret Lundak to authorize only the clarification or updating of deposit data.

2. Addition Of The Reference To The Deposit Is Not New Matter Under 35 USC §132

Second, Applicants respectfully submit that the position taken in the Decision (p. 9-11), that amendment of the '339 specification is improper because it may constitute new matter and thus be precluded by 35 USC §132, is contrary to the controlling law.

In In re Lundak, 773 F.2d 1216 (Fed. Cir. 1985), the Federal Circuit was faced with the issue of whether a post-filing date deposit of a biological material and amendment of the

specification to reference such deposit was new matter under 35 USC §132. The court concluded that the post-filing date deposit was proper and that "the insertion of deposit or data after filing is not new matter under 35 USC §132." Lundak, 773 F.2d at 1223 (emphasis added). The court explained:

> An accession number and deposit date add nothing to the written description of the invention. They do not enlarge or limit the disclosure. This is not the shape of new matter against which section 132 was designed to guard.

Lundak, 773 F.2d at 1223 (emphasis added). See also MPEP §2406.01. ("The [Lundak] court further held that the addition of information designating the depository, accession number, and deposit date of the deposited cell line in ATCC after filling date did not violate the prohibition against new matter in 35 USC 132.")

In light of the Federal Circuit's explicit holding that addition of a reference to a post-filing date deposit does not constitute new matter, Applicants respectfully submit that addition of a reference to the deposit of clone λBH-10, made two months before the filing date, cannot be considered new matter.

Applicants note that, based on a decision in Chiron v. Abbott Laboratories, Civil Action No. 93-4380 MHP, reported at 902 F. Supp. 1103 (N.D. Cal. 1995), the Decision questioned whether the '339 specification satisfied the enablement and best mode requirements of 35 USC §112. Applicants respectfully submit that reliance on the decision in the Chiron v. Abbott litigation is improper for several reasons. First, neither Applicants nor their

assignees (Centocor, Inc. and the National Institutes of Health) are parties to this action. Consequently, Applicants had no opportunity to be heard or to defend against Chiron's allegations regarding their specification. Thus, any decision in the Chiron-Abbott litigation cannot be res judicata as to Applicants, nor may it preclude or estop Applicants from defending their applications. Additionally, as stated in the opinion of the Northern District of California Court, the Judge considered the record before her "quite weak" and made her decision without benefit of expert testimony on these issues.

Second, the issue of whether the '339 application is enabling without reference to the deposit of clone λBH-10 is irrelevant to the instant petition. 37 CFR §1.802(b) states that "[i]f a deposit is necessary, it shall be acceptable if made in accordance with these regulations." As discussed supra, section 1, the deposit of clone λBH-10 was made in full compliance with the deposit rules, which do not require proof that a specification is enabling without the deposit as a prerequisite for allowing the post-filing date addition of a reference to a deposit.[2] Moreover, the discussion in Lundak about whether the specification as filed was enabling was necessitated by Lundak's appeal of the rejection

---

[2] Indeed, 37 CFR §1.802(c) states:

> The reference to a biological material in a specification disclosure or the actual deposit of such material by an applicant or patent owner does not create any presumption that such material is necessary to satisfy 35 USC 112 or that deposit in accordance with these regulations is or was required.

of his claims under 35 USC §112, not because it was a requirement to allow the addition of a post-filing date reference to his specification. See e.g., 773 F.2d at 1220.

Because the deposit of λBH-10 is in full compliance with the governing law and the addition of a reference to the pre-filing date deposit of λBH-10 "is not the shape of new matter against which section 132 was designed to guard," Lundak, 772 F.2d at 1223, Applicants respectfully submit there is no need to examine the '339 application for new matter, and thus, 35 USC 132 does not bar entry of this amendment.[3] Indeed, Applicants submit that the proposed amendment is akin to the request to add a section 120 reference previously allowed by the Office of Petitions and is fully within the scope of amendments authorized by Sampson v. Commissioner of Patents, 195 U.S.P.Q. 136 (D.D.C. 1976).[4] As such, Applicants respectfully request that the amendment be entered.

---

[3] Contrary to the Decision at p. 8, Applicants are not seeking "to change the invention disclosed or to introduce a concept not previously present in the specification of an abandoned application or to continue prosecution of an abandoned application". Rather, Applicants are seeking to add the depository data for an HIV clone which was specifically identified in the specification as filed and was deposited two months prior to the filing date. See Wong-Staal Decl. ¶¶ 4, 10. As discussed supra, in view of the PTO rules and procedure and Federal Circuit precedent, such an amendment is not new matter and does not require examination under 35 U.S.C. §132.

[4] Applicants respectfully submit that Dart Industries, Inc v. Banner, 636 F.2d 684, 687-688 (D.C. Cir. 1980), cited in the Decision at p. 10, is inapposite because reference to a biological material specifically identified in the specification is not new matter under Lundak and the PTO Deposit rules.

CONCLUSION

Applicants respectfully request that the March 29, 1996 Decision be reconsidered and that the petition be granted and the amendment to the '339 application be entered to protect Applicants' patent rights.

AUTHORIZATION

The Assistant Commissioner is hereby authorized to charge any additional fees which may be required in this application, including a petition fee, to Deposit Account No. 13-4500, Order No. 1436-4094.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

By: /William S. Feiler/
William S. Feiler
Reg. No. 26,728

Of Counsel:

Eugene Moroz
M. Caragh Noone
MORGAN & FINNEGAN, L.L.P.
345 Park Avenue
New York, New York 10154
(212)758-4800
(212)751-6849 (FAX)

James C. Haight
NATIONAL INSTITUTES OF HEALTH
Office of Technology Transfer
Suite 325
6011 Executive Blvd.
Rockville, MD 20852
(301) 496-7056

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Nancy Chang, et al.

Serial No. : 06/659,339

Filed : October 10, 1984

For : CLONING AND EXPRESSION OF HTLV-III DNA

ATTN: Mr. Brian Hearn
Assistant Commissioner for Patents
Box DAC
Washington, D.C. 20231

SUPPLEMENT TO REQUEST FOR RECONSIDERATION OF THE MARCH 29, 1996 DECISION DISMISSING APPLICANTS' PETITION PURSUANT TO 37 CFR §1.182 TO ADD A REFERENCE TO A PRE-FILING DATE DEPOSIT

Sir:

Applicants would like to thank Special Projects Examiner Brian Hearn for the courtesies extended in the Interview on May 28, 1996. The following additional comments are respectfully offered in support of their May 28, 1996 Request for Reconsideration of the March 29, 1996 decision dismissing their petition to insert a reference to the pre-filing date deposit of recombinant phage clone λBH-10, which is specifically identified in the specification of U.S.S.N. 06/659,339 ("the '339 application").

Applicants submit that for the reasons set forth below, as well as those in their May 28, 1996 Request for Reconsideration and their Petition, amendment of U.S.S.N. 06/659,339, now abandoned, to add the reference to the deposit is appropriate pursuant to the governing case law, the PTO deposit rules, 37 CFR

§§1.801-1.809, and the Manual of Patent Examining Procedure ("MPEP").

1. The Reference to the Deposit of Clone λBH-10 Is Not New Matter Under 35 U.S.C. §132

As explained in the Request for Reconsideration in In re Lundak, 773 F.2d 1216, 227 U.S.P.Q. 90 (Fed. Cir. 1983), the Federal Circuit held that a post-filing date deposit and of a biological material "is not new matter under 35 U.S.C. §132" and is permissible where the biological material is identified in the specification as filed. Lundak, 773 F.2d at 1223, 227 U.S.P.Q. at 96 (emphasis added). The Lundak court rejected the Board's argument "that both the deposit and its accession number are new matter," Lundak 773 F.2d at 1223, 227 U.S.P.Q. at 95, and explained:

> An accession number and deposit date add nothing to the written description of the invention. They do not enlarge or permit the disclosure. This is not the shape of new matter against which section 132 was designed to guard.

Lundak, 773 F.2d at 1273, 227 U.S.P.Q. at 95 (emphasis added.)

In the instant situation, applicants are seeking to add the accession number and date of the pre-filing date deposit of clone λBH-10, which, as discussed in the Request for Reconsideration, the Amendment, and the Declaration of Dr. Wong-Staal, is specifically identified and described in the '339 specification as filed on page 3, lines 28-30, page 8, lines 33 to

end, and page 9, lines 1-8. Thus, the reference to the deposit of clone λBH-10, is not new matter under Lundak.

Applicants' argument that Lundak permits post-filing date deposits of biological materials identified in the specification as filed is supported by Ex parte DeCastro, 28 U.S.P.Q. 2d 1391 (Bd. Pat. App. & Int. 1993). In DeCastro, the Board, noting that in Lundak "a subsequent deposit of biological material described in the original application was determined to be sufficient to comply with the enablement requirement of 35 U.S.C. §112, first paragraph", held that post-filing date deposit of a biological material that was not identified or described in the specification could not rectify the enablement problems that were found to exist. DeCastro, 28 U.S.P.Q. 2d at 1394 (emphasis added). Thus, the DeCastro case makes clear that Lundak is properly understood to permit post-filing date deposits (and references to the deposits) of biological materials identified in the specification as filed.

Moreover, the Federal Circuit's decision in Lundak, that a post-filing date deposit or reference to a deposit of a biological material identified in the specification is not new matter, is supported by the PTO deposit rules, the MPEP and pertinent case law.[1] The deposit rules make clear that the

---

[1] Pursuant to Mr. Hearn's request at the interview, applicants have obtained a copy of the Lundak application. The abstract contains a statement manifesting an intent to make a deposit. However, applicants submit that this statement is irrelevant to the holding in Lundak and the decision on the instant petition. Indeed, the Lundak court broadly held "that the insertion of depository data after filing is not new matter under 35 U.S.C. §132." Lundak 773 F.2d at

biological material, not the intent to deposit the material, must be referenced in the specification as filed:

> Whenever a biological material is specifically identified in an application for patent as filed, an original deposit thereof may be made at any time before filing the application for patent or, subject to 1.809, during pendency of the application for patent.

37 C.F.R. §1.804(a) (emphasis added).

The MPEP explains that the fact that the biological material to be deposited is referenced in the specification as filed provides an antecedent basis for the deposit, preventing it from being new matter:

> 37 C.F.R. §1.804(a) specifies not only a permissible time for making an original deposit, but also specifies that the biological material deposited must be specifically identified in the application as filed. The requirement of a specific identification is consistent with the description requirement of the first paragraph of 35 U.S.C. 112 and provides an antecedent basis for the biological material which either has been or will be deposited before the patent is granted.

MPEP §2406.01 (emphasis added). Indeed, the MPEP makes clear that while a post-filing date deposit of an identified biological material does not implicate the prohibition against new matter,

---

1223, 227 U.S.P.Q. at 96. Moreover, as discussed infra, the deposit rules (37 CFR §1.804) and MPEP (§2404.03 and §2406.01) make clear that the biological material, not the intent to deposit, must be referenced in the specification as filed. In short, both Lundak and the PTO rules and procedure require only that the biological material be identified in the application as filed.

addition of a reference to a previously unidentified biological material is potentially prohibited by 35 U.S.C. §132:

> It should be noted, however, that reference to a biological material present in an application upon filing, may form the basis for making a deposit, where required, after the filing date of a given application, but that the reference to the biological material itself cannot be added after filing without risking the prohibited introduction of a new matter. 35 U.S.C. 132

MPEP §2404.03 (emphasis added)

Additionally, decisions of the Board of Patent Appeals and Interferences as well as the Court of Customs and Patent Appeals make clear that addition of information which clarifies or describes inherent characteristics or properties of the disclosure, such as the depository information sought to be added here, is not new matter barred by 35 U.S.C. §132. In Application of Reynolds, 443 F.2d 384, 170 U.S.P.Q. 94 (C.C.P.A. 1971), the Court of customs and Patent Appeals explained:

> By disclosing in a patent application a device that inherently performs a function, operates according to a theory, or has an advantage, a patent applicant necessarily discloses that function, theory or advantage even though he says nothing concerning it. The application may later be amended to recite the function, theory or advantage without introducing prohibited new matter.

443 F.2d at 1293, 170 U.S.P.Q. at 98.

Similarly, in Tektronix, Inc. v. United States, 445 F.2d 323 (Ct. Cl. 1971), the Court of Claims held that clarifying or making explicit information which was implicit in the specification as filed did not violate the prohibition against new matter of 35

U.S.C. §132. Tektronix, 445 F.2d at 326-327. The Tektronix patentee amended a drawing to show a previously omitted component of the claimed device and amended the specification to explain and describe the added component. The court concluded that these "clarifying amendments" were not new matter and that by their addition, "an implicit teaching was made explicit." Tektronix 445 F.2d at 349. A copy of this decision is attached as Exhibit A.

In Ex parte Doushkess, 47 U.S.P.Q. 525 (Pat. Off. Bd. of App. 1940) the Board held that addition of disclosure regarding the solubility of the claimed compound was not new matter although this property alone distinguished the invention over certain prior art. The Board explained that because solubility "was an inherent characteristic of the [claimed] mixture," addition of the disclosure regarding this property was not new matter. Doushkess, 47 U.S.P.Q. at 525-526. See also Ex parte Davisson and Finlay, 133 USPQ 400, 402 (Pat. Off Bd. App. 1958) (noting that entry of an amendment which described inherent properties or characteristics of the claimed substance, such as its optical rotation data and spectroscopic characteristics, was proper).

Clearly, addition of information concerning the pre-filing deposit of a HIV clone that was identified and described in the specification as filed simply serves to clarify and make explicit information that was inherent in the disclosure at the filing date. Thus, the amendment sought is not new matter according to these decisions.

In short, the governing case law and PTO rules and procedure all make clear that a post-filing date reference to a biological material specifically identified in the specification as filed is permissible and is not new matter under 35 U.S.C. §132.

2. The Amendment Sought Is Permissible In an Abandoned Application

The PTO and courts have permitted applicants to amend abandoned applications to add or correct technical information. For example, in In re Schmidt, 130 U.S.P.Q. 404 (C.C.P.A. 1961), amendment of an abandoned application to correct the inventorship was sanctioned. Schmidt, 130 USPQ at 410 ("We hold, therefore, that appellant was entitled under section 116 to correct the error in the intermediate [abandoned] application filed in the names of joint inventors").

Similarly, in Sampson v. Commissioner of Patents, 195 U.S.P.Q. 136 (D.D.C. 1996), the applicant was permitted to amend an abandoned application to include "technical information". In this case the technical information consisted of the filing dates and relationship to applications applicant was seeking benefit of. Sampson 195 U.S.P.Q. at 137.

In the instant petition, applicants seek to add only technical information regarding the pre-filing deposit of clone λBH-10 (e.g. the deposit date, accession number and identification of the depository). As discussed above, none of this information is new matter. Thus, the amendment sought is akin to those allowed in Sampson and Schmidt. As such, applicants respectfully submit it

is proper under the case law and PTO rules and procedure and should be entered.

3. Granting The Petition Will Not Create A Dangerous Precedent

In the interview held with Mr. Hearn on May 28, 1996, a concern was expressed that if the PTO were to permit the applicants to amend the '339 application, a plethora of similar requests could be expected which would inundate the Office of Petitions. Applicants respectfully submit that this concern is misplaced.

First, the Office of Petitions presumably has the option not to publish the decision to grant the instant petition. Second, even assuming such a decision was published, it would clearly be limited to the specific and highly unusual factual situation present in the '339 application. Applicants submit that the current situation, in which a deposit of a recombinant clone made over two months before the filing date of a now abandoned application was inadvertently not referenced in the specification as filed, although the clone itself was identified and described in the specification, is unlikely to ever arise again, much less in numbers sufficient to overwhelm the Office of Petitions.

Thus, any decision granting the instant petition would necessarily be limited to facts so unique they are unlikely to occur again. In short, applicants submit that the instant petition, which as discussed above is proper under the pertinent case law and PTO rules and procedure, and should be granted.

4. Conclusion

Applicants respectfully request that the March 29, 1996 Decision be reconsidered and that the petition be granted and the amendment to the '339 application be entered to more fully protect Applicants' patent rights.

5. Authorization

The Assistant Commissioner is hereby authorized to charge any additional fees which may be required in this application, including a petition fee, to Deposit Account No. 13-4500, Order No. 1436-4094.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

By William S. Feiler
William S. Feiler
Reg. No. 26,728

Mailing Address:

MORGAN & FINNEGAN, L.L.P.
345 Park Avenue
New York, New York 10154
(212) 758-4800
(212) 751-6849

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Paper No. 10

MORGAN & FINNEGAN
345 PARK AVENUE
NEW YORK, NEW YORK 10154

DOCKET DEPT
AUG 27 1997
MORGAN & FINNEGAN

COPY MAILED
AUG 25 1997
OFFICE OF PETITIONS
A/C PATENTS

In re Application of :
Nancy Chang :
Application No. 06/659,339 : ON PETITION
Filed: October 10, 1984 :
Attorney Docket No. CTR84-7 :

This is a decision on the renewed petition under 37 CFR 1.182, filed May 28, 1996 to amend the above-identified abandoned application by insertion of a reference to the pre-filing date deposit of a recombinant phage clone, λBH-10, harboring HTLV-III DNA.

The petition is DENIED.

BACKGROUND

The instant application was filed October 10, 1984, naming Nancy Chang as sole inventor.

On May 16, 1986, papers were filed seeking to amend the inventive entity to include Flossie Wong-Staal, and James Gallo, which was never treated by the examiner during the pendency of the application.

On December 15, 1986, an office action was mailed which set a three month period for response, and which *inter alia*, rejected all the elected claims under 35 USC 112 first paragraph, as lacking an adequate written description of the invention and for failing to provide an enabling disclosure, and also noted that "The invention appears to employ novel microorganisms, such as OmpA and pHR100, which were not deposited. It is not clear if the written description is sufficiently clear to avoid the need for a deposit, which must meet the criteria set forth in MPEP 608.01(p)(C)."

As no response was received within the period set and no extensions of time were requested, this application became abandoned March 16, 1987.

A Notice of Abandonment was mailed July 20, 1987.

During litigation not directly involving the instant inventors and assignee, reported as Chiron Corporation v. Abbott Laboratories, 902 F.Supp. 1103 (N.D. Cal. 1995), findings of law were made regarding the (in)sufficiency of the disclosure of the instant specification, under 35 USC 112, first paragraph, and findings of fact including that the specification as filed made no reference to any deposit of biological material.

On February 22, 1996, a petition was filed under 37 CFR 1.182 seeking to (1) insert a reference under 35 USC 120 to application No. 06/643,306, which was pending from August 20, 1984 through July 14, 1987, and (2) insert a reference to the pre-filing date deposit of a recombinant phage clone, λBH-10, harboring HTLV-III DNA. Petitioner relied upon, respectively, Sampson v. Commissioner of Patents, 195 USPQ 136 (D.D.C. 1976), and In Re Lundak, 773 F.2d 1216, 227 USPQ 90 (Fed. Cir. 1985) as authorities for the proposed amendments.

On March 29, 1996, a decision was mailed which granted the first requested item, and dismissed the second, noting that the two issues were independent, and separately decidable.

The instant petition, renewed only with respect to the second item, was filed May 28, 1996.

Petitioner continues to assert that (1) the requested insertion of a reference to the deposit is proper as the biological material is specifically identified in the specification as filed, and (2) the insertion of the reference is not new matter. As to the first issue, petitioner further submits that there is no requirement in the rules, examining procedures, or in Lundak, that a specification as filed must refer to a deposit of a biological material.

STATUTE AND REGULATIONS

35 USC 112, first paragraph requires that:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

35 USC 114 provides in pertinent part:

When the invention relates to a composition of matter, the Commissioner may require the applicant to furnish specimens or ingredients for the purpose of inspection or experiment.

35 USC 131 states

The Commissioner shall cause an examination to be made of the application and the alleged new invention; and if on such examination it appears that the applicant is entitled to a patent under the law, the Commissioner shall issue a patent therefor.

35 USC 132 states:

Whenever, on examination, any claim for a patent is rejected, or any objection or requirement made, the Commissioner shall notify the applicant thereof stating the reasons for such rejection or objection or requirement, together with such information and references as may be useful in judging of the propriety of continuing the prosecution of his application; and if after receiving such notice, the applicant persists in his claim for a patent, with or without amendment, the application shall be reexamined. **No amendment shall introduce new matter into the disclosure of the invention.** (emphasis added)

35 USC 133 states:

Upon failure of the applicant to prosecute the application within six months after any action therein, of which notice has been given or mailed to the applicant, or within such shorter time, not less than thirty days, as fixed by the Commissioner in such action, the application shall be regarded as abandoned by the parties thereto, unless it be shown to the satisfaction of the Commissioner that such delay was unavoidable.

37 CFR 1.802(c) states:

The reference to a biological material in a specification disclosure or the actual deposit of such material by an applicant or patent owner does not create any presumption that such material is necessary to satisfy 35 U.S.C. 112 or that deposit in accordance with these regulations is or was required.

37 CFR 1.804(a) states:

Whenever a biological material is specifically identified in an application for patent as filed, an original deposit

thereof may be made at any time before filing the application for patent or, subject to 1.809, during pendency of the application for patent.

37 CFR 1.808 states that:

(a) A deposit must be made under conditions that assure that:

(1) Access to the deposit will be available during pendency of the patent application making reference to the deposit to one determined by the Commissioner to be entitled thereto under 1.14 and 35 U.S.C. 122, and

(2) Subject to paragraph (b) of this section, all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of the patent.

37 CFR 1.809 provides in pertinent part:

(a) The examiner shall determine pursuant to 1.104 in each application for patent, application for reissue patent or reexamination proceeding if a deposit is needed, and if needed, if a deposit actually made is acceptable for patent purposes. If a deposit is needed and has not been made or replaced or supplemented in accordance with these regulations, the examiner, where appropriate, shall reject the affected claims under the appropriate provision of 35 U.S.C. 112, explaining why a deposit is needed and /or why a deposit actually made cannot be accepted.

(b) The applicant for patent or patent owner shall respond to a rejection under paragraph (a) of this section by --

(1) In the case of an applicant for patent, making an acceptable original or replacement or supplemental deposit or assuring the Office in writing that an acceptable deposit will be made on or before the date of payment of the issue fee, or, in the case of a patent owner, requesting a certificate of correction of the patent which meets the terms of paragraphs (b) and (c) of 1.805, or

(2) Arguing why a deposit is not needed under the circumstances of the application or patent considered and/or why a deposit actually made should be accepted. Other replies to the examiner's action shall be considered nonresponsive. The rejection will be repeated until either paragraph (b)(1) of this section is satisfied or the examiner is convinced that a deposit is not needed.

OPINION

The proposed amendment, in this case, does not adequately comply with the applicable rules and statutes, to permit insertion.

Petitioner asserts that the referenced material of the deposit is identical to that disclosed in the specification, and includes a declaration by Dr. Flossie Wong-Staal (Staal) to that effect. As such, petitioner asserts, the deposit is in compliance with the deposit rules 37 CFR §§ 1.801-1.809 (specifically 37 CFR 1.804(a)), and MPEP § 2400, and therefore, the proposed insertion is proper. Petitioner's contentions are without merit, as the deposit has not been made in accordance with the deposit rules and patent statutes. As such, the mere assertion of identity between the material deposited by Staal, and that disclosed in the specification, is immaterial to the requested insertion of a reference to the material deposited by Staal.

Rather, the meaning of 37 CFR 1.804(a) is that a biological material may be acceptably deposited before filing an application if an adequate antecedent basis for the deposit subsequently exists in that application *as filed*. 54 FR 34864 (August 22, 1989). However, as set forth in the examiner's action mailed December 15, 1986, and as held in Chiron, this application, *as filed*, did not comply with 35 USC 112, and as such, did not provide adequate antecedent basis for referencing a deposit. Chiron held that this specification *as filed* did not specifically mention the clone sought to be inserted herein (at 1126), and further, failed to disclose the means for obtaining the necessary starting material to practice the invention (at 1129). It follows that the specification *as filed* did not provide adequate antecedent basis for the deposit, and, as such, cannot now be properly referenced to the deposit. Further, as noted in 54 FR 34869, "[I]t must be clear from the application as filed that the invention claimed and described in the specification "was fully capable of being reduced to practice (i.e., no technological problems, the resolution of which would require no more than ordinary skill and reasonable time, remained in order to obtain an operative, useful process)[.]", a situation clearly not present here. Petitioner' reliance on 37 CFR 1.802(c) is inapt, as the deposit rules do not address the substantive issue of whether a deposit is required under any particular set of facts. 54 FR 34864. Rather, the examiner addresses that substantive issue, as "reference to a biological material cannot be added to a specification without risking the prohibited introduction of new matter (35 U.S.C. 132)." 54 FR 34875-34876. Petitioner's further reliance on 37 CFR 1.809 is similarly misplaced, as the regulation speaks to procedures that will be used by the examiner to address deposit issues. 54 FR 34878. However, the examiner has no procedural authority with respect to

an abandoned application. Lorenz v. Finkl, 333 F.2d 885, 891, 142 USPQ 26, 30 (CCPA 1964).

As noted in the previous decision, this application was pending from October 10, 1984, through March 15, 1987. Petitioner appears to overlook the fact that, as noted in MPEP 2402:

> "The rules are effective for all applications filed on or after January 1, 1990,...except that deposits made prior to the effective date which were acceptable under the then current practice will be acceptable in such applications and proceedings. Since most of the provisions of the rules reflect policy and practice existing prior to January 1, 1990, little change in practice or burden on applicants for patent... has occurred. Applicants and patent owners are encouraged to comply with these rules even if their applications and reexamination proceedings were filed prior to January 1, 1990. The current text of MPEP § 608.01(p)(C) is controlling practice for applications filed prior to January 1, 1990"

It should be noted that the practice for making an acceptable deposit prior to January 1, 1990, specified:

"When the invention depends on the use of a microorganism which is not so known and readily available, applicants must take additional steps to comply with the requirements of § 112. [The court in] In re Argoudelis, et al., 168 USPQ 99 (CCPA, 1970), accepted a procedure for meeting the requirements of 35 U.S.C. 112. Accordingly, the Patent and Trademark Office will accept the following as complying with the requirements of § 112 for adequate disclosure of the microorganism required to carry out the invention:

> (1) the applicant, no later than the effective U.S. filing date of the application, has made a deposit of the microorganism in a depository ... **under conditions which assure (a) that access to the culture will be available during pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 CFR 1.14 and 35 USC 122 and (b) that all restrictions on the availability to the public of the culture will be irrevocably removed upon the granting of the patent;**
>
> (2) such **deposit is referred to in the body of the specification as filed and is identified by deposit number, name and address of the depository,** and the taxonomic description to the extent available is included in the specification;

> (3) the applicant or his assigns has provided assurance of permanent availability of the culture to the public through a depository meeting the requirements of (1). Such assurance may be in the form of an averment under oath or by declaration by the applicant to this effect.
>
> A copy of the applicant's contract with the depository may be required by the examiner to be made of record as evidence of making the culture available under the conditions stated above." (emphasis added)[1]

Since the instant specification as filed, and during pendency of the patent application, made no reference to a deposit, *inter alia*, and thus, the examiner was denied awareness of, and access to, the deposit, 35 USC 112 and 114 were not satisfied. Such is not an acceptable deposit procedure, then and now, and moreover, such failed to rise to the circumstances of Lundak. As such, petitioner's reliance upon Lundak is a *non-sequitur*.

Notwithstanding that this application was filed prior to January 1, 1990, petitioner improperly seeks to rely upon the current deposit rules. Assuming, *arguendo*, they are applicable to this application, petitioner has not shown, nor is it apparent, how the current deposit rules can be satisfied in this instance. *E.g.*, 37 CFR 1.808, requires in pertinent part:

> "(a) A deposit must be made under conditions that assure that:
>
> (1) Access to the deposit will be available during pendency of the application making reference to the deposit to one determined by the Commissioner to be entitled thereto under § 1.14 and 35 U.S.C. 122," (emphasis added)

It follows that access to the deposit cannot "be available" (or have been available) to the Office *during pendency* of this application, as required by the examiner in the office action mailed December 15, 1986 [2], the regulations, and statutes (35 USC

---

[1] Lundak, at 1219, 227 USPQ at 92. Lundak's application was pending from March 26, 1981, through June 10, 1986, which compares reasonably with that of the instant application (October 10, 1984 through March 15, 1987) for considering the then current practice. The practice set forth above is that of MPEP § 608.01(p)(C), now more fully codified in the deposit rules.

[2] While petitioner complains that he was not a party to the recent Chiron litigation, the court's holdings of law and

112 and 114), for pending patent applications, Lundak, at 1222, 227 USPQ at 95, as (1) the deposit was not referenced in the instant application during its pendency, much less in the specification as filed, and (2) the deposit was not made "under conditions which assure", during pendency of the instant application, availability to one determined by the Commissioner to be entitled to such, or, after grant of a patent, unrestricted public availability. Id. at 1219, 227 USPQ at 92; Feldman v. Aunstrup, 517 F.2d 1351, 1354, 186 USPQ 108, 112 (CCPA 1975).

As noted in 54 FR 34839, "The Office will treat a deposit not made according to these regulations, however, as if no deposit had been made."

Petitioner has not shown how the requirements of 35 USC §§ 112, 114 for PTO access to a sample of petitioner's material during pendency of this application, and of 35 USC § 112, for public access after grant, were met by petitioner's deposit procedures. Cf. Lundak at 1222, 227 USPQ at 95. The making of a deposit in 1984, and the 1996 attempt to reference that deposit *nunc pro tunc* in an application examined in 1986 and abandoned in 1987 is not seen to be in accordance with the deposit rules, or the patent statutes. As noted in Lorenz, at 889, 142 USPQ at 29:

> "We think it is apparent however, that Congress did not intend that applicant should be able to 'prosecute' his application indefinitely before the Patent Office. An orderly administrative process demands an end to prosecution."

Rather, petitioner is using Chiron as an improper basis for the belated attempt to continue prosecution by responding to the examiner's action of ten years ago, and thereby finesse, *inter*

---

findings of fact regarding this application closely parallel sections of the examiner's action herein dated 10 years earlier. The record does not indicate why petitioner took no timely opportunity to be heard with regard to either situation. As to petitioner's further assertion that the Chiron litigation "may [not] preclude or estop Applicants from defending their applications", petitioner only had that opportunity with respect to this application while it was pending before the Office, and failed to then "defend" it. Cf. Sampson, where that applicant diligently and assiduously defended his rights, such that unusual delay or laches was not a bar to amending that abandoned application under 35 USC 120. Petitioner has failed to cite any authority for "defending" an abandoned application beyond the mere insertion of a § 120 statement, much less after a 10 year hiatus in activity.

alia, the doctrine of laches; the failure to comply with the deposit practice then in effect; the examiner's consideration of their belated response; and the abandoned status of this application. However, this application is abandoned by operation of law, and petitioner has made no attempt to revive this application under 35 USC 133, much less make a showing of unavoidable delay satisfactory to the Commissioner. It follows that petitioner will not be permitted, in the guise of a petition, to prosecute this application indefinitely before the Patent Office, and circumvent an orderly administrative process.

Petitioner's contentions with respect to the new matter issue are also without merit.

As explained in Dart Industries v. Banner, 636 F.2d 684, 688, 207 USPQ 273 at 276 (C.A.D.C. 1973):

> "An addition to a patent specification constitutes new matter when it changes the invention disclosed or introduces a concept not previously present in that specification." [citations omitted].

An amendment to the specification of a pending application must be considered by the examiner for new matter pursuant to 35 USC 132; however, the examination of an application pursuant to 35 USC 131 *et seq*. involves pending applications. Borenz, Id.

Moreover, the fact that the Patent Office permits an amendment to an application without objection thereto as new matter during the examination process is given special deference by the courts. See, Brooktree Corp. v. Advanced Micro Devices, Inc., 977 F.2d 1555, 1573, 24 USPQ2d 1401, 1414 (Fed. Cir. 1992). The requested amendment of this abandoned application by petition would operate to evade the examination of such amendment for new matter pursuant to 35 USC 131 and 132[3], as well as the requirement of the deposit regulations and 35 USC §§ 112, 114 that the material

---

[3] The previous decision permitted petitioner to insert only the technical information required to obtain a benefit under 35 USC § 120, under the authority of Sampson. However, it is well settled that, since a § 120 statement is merely a mechanism for obtaining the benefit of the filing date of an earlier application, which mechanism does not change the invention disclosed in, or introduce a concept not previously disclosed into, the later application, there was no issues(s) of new matter. As such, no examination of this abandoned application, or the amendment, for new matter was required, or evaded. In re Lambrech, 202 USPQ 620 (Comm'r Pat. 1976).

be (or have been) available to the examiner and Office for examination during pendency.

Petitioner challenges the previous decision where it stated that Lundak's specification as filed made reference to a deposit. Nevertheless, the specification *as filed* in Lundak specifically made reference to a deposit, *i.e.*, the concept of deposit was present in the specification as filed. Id. at 1223, 227 USPQ at 95. Lundak erroneously believed, and the specification reflected his belief, that the deposit had been made with the ATCC prior to filing, when in fact the deposit was made about one week later. Note also, Lundak asserted that he deposited the material at his university, and elsewhere, prior to filing. In this light, the court ruled that the subsequent addition of merely the date of deposit and accession number did not constitute new matter.[4] As such, and contrary to petitioner's contentions, Lundak does not authorize that reference to a deposit *per se* may be inserted for the first time into a pending application, much less into an abandoned application. Rather, Lundak merely held that when a specification as filed: otherwise satisfies 35 USC 112, and contains reference to a deposit of biological material; the insertion of an updated date of deposit and accession number to the pre-existing deposit statement in the specification of a pending application does not constitute new matter. Where, as here, the application is not pending, the specification does not satisfy 35 USC 112[5], and does not, as filed, contain a reference to a deposit[6], the conclusion is inescapable that Lundak is inapposite.

While petitioner asserts that Lundak held that the insertion of deposit data is not new matter as prohibited by 35 USC 132, a fair reading of the decision reveals this holding is actually limited to insertion of "[a]n accession number and deposit date" into the deposit statement. Id. at 1223, 227 USPQ at 96. As noted *supra*, the specific reference to the concept of deposit was present in the Lundak specification *as filed*. Therefore, the addition of "[a]n accession number and deposit date" was not a new concept prohibited by 35 USC 132. See also In re Fouche, 439 F.2d 1237, 169 USPQ 429 (CCPA 1971).

Where petitioner argues that as the Staal deposit of λBH-10 complies with the governing deposit regulations, and thus, the

---

[4] 3 D. Chisum, Patents § 7.03[5] (1997).

[5] Chiron, Id. at 1126, 1127.

[6] Chiron, Id. at 1108.

addition of a reference to the deposit of same is not new matter, such is a *non sequitur*. The deposit rules are subservient to the statutory prohibition of the insertion of new matter into a specification. 35 USC 132. Even assuming, *arguendo*, that the deposited material λBH-10 is that disclosed in the specification, the specification never made reference to a *deposit* of the material. Absent such, the proposed amendment does not amount to an acceptable mere change of wording, as it introduces a concept *i.e.*, *deposit*, not previously set forth. Fouche, Id.[7] Rather, the proposed amendment is an incorporation by reference of the deposited material. Ex Parte Maizel, 27 USPQ2d 1662, 1669 (BPAI 1992). However, a specification that fails to comply with 35 USC 112, first paragraph, as filed, may not be thereafter augmented, as such would entail the addition of new matter within the meaning of 35 USC 132. In re Hay, 534 F.2d 917, 189 USPQ 790 (CCPA 1976). Specifically, a post-filing attempt at incorporation by reference is prohibited where such would entail the addition of new matter. Dart, Id.; In Re Hawkins, 486 F.2d 579, 179 USPQ 163 (CCPA 1973).

Even assuming, *arguendo*, that insertion of reference to a deposit is not an incorporation by reference, petitioner has failed to meet his burden of showing where the concept of deposit was set forth with reasonable precision in the specification as filed. Absent such a showing, the proposed introduction of the concept of deposit must be regarded, as a threshold determination, as the proposed, but prohibited, insertion of new matter. Fouche, Id.; Dart, Id.; Hawkins, Id.

Petitioner asserts that the instant request is akin to the § 120 statement authorized in the previous decision under Sampson, in that there is no need to examine a proposed § 120 statement for new matter. However, as noted in the previous decision, Dart makes it clear that insertion of a mere § 120 statement, cannot enlarge or alter the disclosure of an abandoned application, and cannot introduce a concept not previously set forth in that

---

[7] The specification of Fouche, as filed, contained a specific, but incomplete, referencing statement to another application which therein set forth the necessary enabling disclosure of how to make Fouche's starting material. The court held that this was an adequate incorporation by reference such that a subsequent amendment to specify serial number and filing date did not introduce new matter into the specification. Id. Compare, Lundak's as-filed specification which contained a specific reference to a deposit, and the court's ruling that a subsequent amendment adding only the deposit number and deposit date did not introduce new matter into the specification.

disclosure. As such, there is no need to reexamine an abandoned application and a § 120 statement when inserting such into that abandoned specification under the authority of Sampson. In re Lambrech, 202 USPQ 620 (Comm'r Pat. 1976). Absent an equally clear and convincing authority for a threshold determination that insertion of a reference to a deposit, which reference is submitted after the filing, much less the abandonment, of an application, will not enlarge or alter the disclosure of that application, or will not introduce a concept not previously set forth in that application as filed, that proposed amendment, and abandoned application, must be examined for a determination of new matter *vel non*. However, petitioner has overlooked the fact that the all proceedings related to the examination of this application have been terminated since March 16, 1987, and that the examiner has lacked any prodedural authority with respect to this application since that date. Lorenz, supra.

Conclusion

The previous decision has been reconsidered, but for the reasons given above, will not be changed. The request to amend the specification by insertion of a reference to a deposit of any biological material, including λBH-10, is denied.

This abandoned application is being returned to the Files Repository.

Telephone inquiries related to this decision should be directed to Special Projects Examiner Brian Hearn at (703) 305-1820.

Charles Pearson
Patent Legal Administrator
BH/KS/AH

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Nancy Chang et al.

Serial No. : 06/659,339

Filed : October 10, 1984

For : CLONING AND EXPRESSION OF HTLV-III DNA

Assistant Commissioner for Patents
Box DAC
Washington, D.C. 20231

DECLARATION OF FLOSSIE WONG-STAAL, Ph.D.

I, Dr. Flossie Wong-Staal, hereby declare:

1. I am an inventor of the subject matter described and claimed in the above-referenced application.

2. My educational background and professional and research experience are listed in my curriculum vitae, attached as Ex. A.

3. In July 1984, I deposited a recombinant phage clone harboring HTLV-III DNA, designated λBH-10, with the American Type Culture Collection ("A.T.C.C."), 12301 Parklawn Drive, Rockville, MD 20852.

4. The A.T.C.C. filing receipt, attached as Exhibit B, shows that the deposit was received and accepted by the A.T.C.C. on July 30, 1984. The accession number of clone λBH-10 is 40125.

5. The A.T.C.C. filing receipt certifies that the A.T.C.C. is an International Depository Authority established

under the Budapest Treaty and that the deposit was made under the Budapest Treaty.

6. The A.T.C.C. filing receipt also states that clone λBH-10 will be maintained for a period of at least 30 years from the deposit date and at least 5 years after the most recent request for a sample.

7. The A.T.C.C. filing receipt certifies that clone λBH-10 will be made available if "a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. patent is issued citing" λBH-10. Additionally, the A.T.C.C. filing receipt states that the viability of clone λBH-10 has been tested and confirmed.

8. The λBH-10 clone deposited on July 30, 1984 was specifically identified, described and characterized in U.S.S.N. 06/659,339 ("the '339 application"), as filed, on October 10, 1984.

9. In the '339 specification we used the short-hand or abbreviated phrases "lambda$_{10}$ clones" or "λ_{10}" to refer to the λBH-10 clone. This nomenclature refers to the fact that HTLV-III molecular clone BH-10 had been inserted into bacteriophage lambda.

10. The '339 specification, attached as Exhibit C, identifies clone λBH-10 and describes its uses and characterization. For example, the '339 specification states that in one embodiment of the invention, "lambda$_{10}$ clones harboring HTLV-III DNA are cloned from the replicated virus."

(page 8, line 33 to page 9, line 1). Similarly, the '339 specification also describes the characterization of the λBH-10 clone (designated in "λ_{10}" Figures 1a and 1b) at page 9 of the text:

> Cuts are made in the cloned HTLV-III DNA with the restriction enzyme Sst I. (Figure 1a) Because there are two Sst I recognition sites within the LTR of HTLV-III DNA, one LTR region is not present in the cloned DNA sequence removed from the lambda$_{10}$ vector . . . .

(page 9, lines 3-8). The restriction maps presented in Figs. 1a, 1b and 2 of the '339 application further characterize clone λBH-10. Moreover, as described in the '339 specification, we used clone λBH-10 to express and screen recombinantly-produced HTLV-III proteins. See e.g., page 12, lines 1-5 and 11-14.

I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 to Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date 5/14/96

Flossie Wong-Staal, Ph.D.

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☑ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☑ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.